CHAPTER – I

METHOMYL INDUCED TOXICITY IN TESTIS, ACCESSORY REPRODUCTIVE ORGANS AND BIOCHEMICAL CONTENTS IN ALBINO MICE
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INTRODUCTION

Mammalian reproduction is a highly co-ordinated process in which almost all of the biologic resources are mobilized to achieve this critical function. The complex process of reproduction in mammals is influenced by various physical, chemical and nutritional factors (Ellis, 1970; Mann and Lutwak-Mann, 1982; Leathen, 1970). Disruptions of male reproductive function can occur to toxic responses in the endocrine system. Androgen production in the testes is regulated primarily by luteinizing hormone (LH), a gonadotropin released by the pituitary gland. Gonadotropin secretion is in turn regulated by gonadotropin releasing hormone (GnRH), secreted by the hypothalamic portion of the brain. This hierarchal arrangement, where the hypothalamus regulates the pituitary which in turn regulates the gonads, is known as the hypothalamic-pituitary-gonadal axis. The hypothalamic-pituitary-gonadal axis is a toxicological perspective, this arrangement creates even more sites where toxic responses may have an impact on reproduction (Silverman et al., 1994; Hotchkiss and Knobil, 1996; Robert et al., 2000). With this in mind, it is not surprising that some compounds generally considered to affect the central nervous system, can impact Leydig cell function and male reproduction (Robert et al., 2000). Several reports indicate declining sperm counts and increase of these reproductive disorders in some areas during some time periods past 50 years. Disorders of reproduction, toxic hazards to reproductive health and their associated functions have become prominent issues after reports of adverse effects of certain chemicals on reproductive function.

The process of germ cell production in the male, spermatogenesis provides clear examples of how cells may have enhanced susceptibility to certain classes of chemicals at particular times. In a human, it takes about 64 days and 40–50 days in the rodent to produce a mature spermatozoon through this process, which continues throughout adult life (Mandl., 1964, Suresh et al., 2008). The rate of spermatogenesis increases dramatically following puberty until a hundred million or more sperm are produced each day. Spermatogenesis can be equated to a mass production process where constant high rates of production and high quantity of output are the focus. In a biological system, this requires an extremely active, rapidly dividing cellular environment within the testis. Reproductive toxicity may be expressed as alterations in sexual behaviour, decreases in fertility, or loss of the fetus during pregnancy. A reproductive toxicant may interfere with the sexual functioning or reproductive ability of exposed individuals from puberty throughout adulthood. Toxicants that target the...
male reproductive system can affect sperm count or shape, alter sexual behavior, and/or increase infertility.

Testis is the primary male sex organ with two well established function namely, spermatogenesis and steroidogenesis (Stainberger and Stainberger, 1978; Sharpe, 1987). The anterior pituitary participates in the control of both of these functions through its secretion of the gonadotropins, FSH and LH (Smith, 1927). The anterior pituitary-testicular axis is sensitive to a number of exogenous/ environmental factors. Based on the early work of Greep et al., (1936), FSH is held to be responsible for the control of spermatogenesis and LH for testosterone production. The impaired spermatogenesis can occur by indirect or direct mechanisms. Indirect impairment involves interference with hormones that promote spermatogenesis, namely, testosterone and the gonadotropins (Sundram and Witorsch, 1995). Toxicants bring about pathological changes by killing cells through necrosis or apoptosis. The mechanisms differ intensively in terms of the mechanism of action as well as the manifestation of the microscopically discernible organization and the molecular biology of the affected cell (Wyllie et al., 1980; Pesce et al., 1993; Stanley et al., 1995; Averal et al., 1996).

The direct toxic mechanism involves insult to components of the seminiferous tubules, Leydig cells and testicular vasculature. When testes are exposed to environmental chemicals and drugs varying degrees of infertility may result (Lucier et al., 1977; Lee and Dixon, 1978; Steinberger, 1981; Thomas, 1981; Meistrich, 1986; Georgellis et al., 1989). Each of the spermatogenic line cells may be the target for the action of a toxic agent (Foster et al., 1982, 1983; Van Thiel et al., 1983; Chapin et al., 1983, 1984a, b; Sikka et al., 1985; Foster et al., 1986). It has been reported that the germinal epithelium of testis of rodents is sensitive to a wide variety of internal and external factors (Bockelheide et al., 1989). Disorder of development and function of the male reproductive tract have been increased in incidence over the past 30-50 years (Giwereman and Skakeback, 1992). Sperm counts in healthy men around the world have fallen about 50 percent in the last 50 years. Detailed studies of how sperm counts have changed over a time in particular are showed the same pattern with a few exceptions. Researchers hypothesize that exposure to toxic chemicals may be an important cause for the declines (Cox, 1996).
Pesticides cause changes in biochemical constituents of the tissues before histopathological changes. The pesticide induces impairment in metabolism by affecting key enzymes, which are very much essential for detoxification. It is reported that pesticides may induce pathological changes in the testes and different organs of rats and mice (Dikshith and Datta, 1972; Joshi et al., 2003). Many organochlorine and organophosphorous insecticides are toxic to the male reproductive system in animals (Virgo and Bellward, 1975; Shivanandappa and Krishnakumari, 1983). Similarly, Hiremath and Kaliwal (2000) and Jadaramkunti and Kaliwal (2002) have reported that chlorinated pesticides endosulfan and dicrofyl adversely affects the male reproductive system in mice and rats.

Studies on carbamate pesticides revealed varying degrees of intoxication. Administration of carbamate fungicide benomyl in the pubertal and post pubertal rats results in decreased testicular weight and decreased sperm count in the epididymis and vas deferens (Carter et al., 1984). Carbamate insecticide, carbofuran exposure affected epididymis, seminal vesicles, ventral prostate, coagulatory gland with decreased total sperm count and increased sperm abnormalities in rats (Pant et al., 1997). Another carbamate insecticide, carbosulfan at ip dose 5 mg/kg in rat caused increased sperm head abnormalities without affecting the total count of sperms at 24 and 48 hours of treatment, but the same treatment caused increased chromosomal aberration observed in bone marrow cells (Topktas et al., 1996; Giri et al., 2002). Kackar et al., (1999) found that chronic exposure to mancozeb in male rats produced a significant testicular dysfunction, as indicated by a marked reduction in serum testosterone level and sperm count. Goad and coworkers (2004) also demonstrated decreased serum testosterone levels in carbofuran-treated male rats due to reduced biosynthesis and release.

Rani et al., (2007) evaluated the carbaryl exposure showed distorted shape of seminiferous tubules, disturbed spermatogenesis and accumulation of cellular mass in the lumen of tubules, oedema of the interstitial spaces and loss of sperms of varying degrees in testes. In another study of the sperm samples found that, the number of sperm abnormalities was increased in workers who were being exposed to carbaryl (Wyrobek et al., 1981). Pant et al., (1995) have reported carbofuran that was administered orally to adult rats caused a dose dependent decrease in the weight of epididymides, seminal vesicles, ventral prostate and coagulating glands and decreased sperm motility, reduced epididymal sperm count along with increased morphological abnormalities in head, neck and tail regions of spermatozoa.
Carbaryl also causes oedema, congestion, damage to Sertoli cells and germ cells, along with the accumulation of cellular debris and presence of giant cells in the lumen of a few seminiferous tubules which showed disturbed spermatogenesis with the higher doses of carbofuran (Nasir et al., 2008).

Recently, Ksheerasagar and Kaliwal (2010) reported that carbamate fungicide mancozeb affects spermatogenesis and inhibition was reflected by significant decrease in number of spermatogenic cells and sperms and there was significant decrease in the weight of testes, prostate and Cowper's glands. Mancozeb irrespective of dose and duration caused significant decrease in the level of protein in liver and glycogen in the testis, liver and kidney and a significant increase in the level of total lipids in testis (Ksheerasagar and Kaliwal, 2003). Mahgoub and EI-Medany (2000) reported that the rat treated with methomyl orally (17 mg/ kg in saline) daily for two months caused a significant decrease in the level of testosterone was observed in the intoxicated animals, while the levels of FSH, LH and prolactin were significantly increased. Further, histopathological studies of the intoxicated rat testis revealed variable degrees of degenerative changes in the seminiferous tubules up to total cellular destruction and they concluded that subchronic exposure to methomyl insecticide has an obvious deleterious effect on rat testes.

Pesticides are known to induce oxidative stress by excess generation of free radicals, especially reactive oxygen species and reactive nitrogen species, and alteration in antioxidants and the scavenging system, causing lipid peroxidation (Banerjee et al., 1999; Dettbarn et al., 2001; Gupta et al., 2001a, 2002b; Etemadi-Aleagha et al., 2002). The possible mechanism for the loss of testicular and sperm function due to high oxidative stress has been shown to involve excessive generation of ROS (Aitken and Clarkson, 1987). Exposure to types of environmental contaminants can enhance this oxidative process both by increasing generation of free radicals and by decreasing antioxidant potential and thus causing Gonadal damage (Sikka et al., 1995).

Several studies have confirmed that oxidative stress is induced by pesticides in rats (Gultekin et al., 2000; Gupta et al., 2001a; Akhgari et al., 2003) and humans (Banerjee, 2001; Ranjbar et al., 2002). Banks and Soliman (1997) have shown that methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl) induces lipid peroxidation and glutathione depletion in rats due to a significant increase in serum hydroperoxides and a
significant decline in hepatic reduced glutathione (GSH) levels. In addition, N,N-diphenyl-p-phenylenediamine (DPPD) and 21-aminosteroid (U74389G) blocked benomyl-induced lipid peroxidation and GSH depletion, thus confirming that in vivo toxicity of benomyl may be associated with increased oxidative stress to cellular membranes and that some degree of protection against this toxicity could be afforded by antioxidants. Such disturbance in the balance resulting in increased oxidative stress can influence male infertility. Benomyl may induce permanent testicular damage and a decrease in sperm production (Hess and Nakai, 2000). It has been reported that the carbamate pesticides carbendazim and pyrrolidine dithiocarbamate affects cellular antioxidative mechanism and thereby cause testicular damage (Rajeshwary et al., 2007; Ilibey et al., 2009). Recently, Ozden and Alpertunga (2010) reported that N-methylcarbamate insecticide methiocarb induces lipid peroxidation (LPO) in tissues of male Wistar rats following single and repeated oral exposures. LPO was significantly increased and GSH level was significantly decreased following higher doses in liver, kidney, brain and testis, and it is suggested that methiocarb toxicity is associated with oxidative stress.

There are several possible mechanisms for the antigonadal actions of carbamates: they may exert a direct inhibitory action on the testis; they may affect pituitary, causing changes in gonadotropins concentrations and thus spermatogenic impairment; or they may change the concentration of neurotransmitters. Reports regarding effect of methomyl on testis are scanty. Therefore, in the present study, changes in the biochemical, enzymatic, hormonal milieu, histologic and histometric evaluation of testis and epididymis after exposure to methomyl were investigated.
MATERIALS AND METHODS

Animals

Laboratory bred adult virgin Swiss albino mice were used in the experiments. Mice aged 90 days old weighing between 25-30g were used. The mice were maintained in the P.G. Department of Studies in Zoology, Karnataka University, Dharwad. Mice breed quite normally, almost throughout the year and permitted through local ethical committee. They were housed in separate polypropylene cages containing sterile paddy husk as bedding material. The mice were provided with standard mice pellet diet “Gold Mohar” (Hindustan Liver Company, Mumbai) and water ad libitum. The mice were maintained under normal day/night schedule (12 L: 12 D) at room temperature 25 ± 2°C. Animals were randomly divided into control and four treatment groups (Distilled water vehicle is served as controls). Each group consisting of 10 mice in each experiment and Body weight was recorded daily throughout the experiments.

Insecticide

The sample of methomyl (Lannate® 40% SP) used in experiments was commercial carbamate insecticide supplied by E.I. Dupont India Pvt. Ltd., Haryana obtained from the local company’s market containing 40% (w/w) [methomyl (a.i) 40%, inert ingredient (silica) 6%, embittering agent (sucrose octaacetate) 0.4%, emulsifiable oil (sun spray 9 E oil) 6.5%, inert base 47.1%]. The mouse oral LD_{50} for methomyl is 10 mg/ kg body weight (Baron, 1991).

Experiment I

The graded doses of methomyl 1, 2, 3 and 4 mg/kg body weight/ day administered orally for 30 consecutive days. The experiment was designed to determine the effective dose of methomyl on testis, accessory reproductive organs, biochemical contents of testis and epididymis, changes in hormonal milieu and oxidative stress parameters.

Experiment II

This experiment was designed to find out the temporal effect of an effective dose 4 mg/kg body weight/ day. Methomyl was administered orally for 5, 10, 20 and 30 days.
All the experimental animals were autopsied by cervical dislocation on 31st day or 24 hrs after the terminal exposure. The blood was collected from cardiac puncture. Serum was stored at -20°C for hormone assays. The testes and accessory reproductive organs were dissected out and weighed to the nearest milligrams in digital weighing balance (vibra) and were used for histologic and biochemical estimations such as DNA, RNA, protein, glycogen, cholesterol and sialic acid and activity of enzymes SDH, LDH, 3β-HSD, 17β-HSD, Na⁺-K⁺ATPase, Ca⁺⁺ATPase, Mg⁺⁺ATPase, ACP, AKP and oxidative stress parameters in the testes and epididymis as per the methods mentioned.

Histology and histometry

The testes were fixed in aqueous bouin's fluid for 24 hrs. and dehydrated by placing them in 30%, 50%, 70%, 90% and 100% alcohol gradations and cleared in benzene, embedded in paraffin wax. Sections of 5 μm thickness were obtained and stained with haematoxylin-eosin (Humason, 1979). From each testis 10 sections were randomly selected for histologic and histometric observations were made with a calibrated ocular and stage micrometer. In each section ten seminiferous tubules exhibiting round shape between II to VIII stages were selected in accordance with the criteria given by Leblond and Clermont (1952). The seminiferous tubules were examined for counting the different spermatogenic cells and Leydig cells lying around them. The diameter of spermatogenic cells and Leydig cells were determined after 1000 observations of particular cell types/ testis from each animal of control and treated groups. Spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and Leydig cells were identified based on the findings of earlier investigators (Oakberg, 1956; Gordner, 1966; Dym and Fawcett, 1971; Fawcett et al., 1973) as reviewed by deKrester and Kerr (1994). The values were expressed as number and diameter of spermatogenic and Leydig cells per seminiferous tubules.

Biochemical estimations

Estimation of Nucleic acids

Nucleic acids were extracted from the tissues by the method described by Schneider (1957).

Principle: Nucleic acids are separated from the other tissue components by exploiting their property of preferential solubility in hot trichloro acetic acid (TCA) solution.
Known weight of tissue was rinsed finely and homogenized in distilled water, to homogenate 5 ml of 10% ice cold TCA was added and was kept in an ice bath for 30 minutes to allow complete precipitation of proteins and nucleic acids. The mixture was centrifuged and the precipitate was later treated with absolute alcohol and centrifuged to remove lipid materials. The precipitate free of lipids was suspended in required amount of 5% TCA and placed in a water bath maintained at 90°C for 15 mins with occasional stirring which facilitates the quantitative separation of nucleic acids from the precipitated proteins. This was centrifuged and the supernatant was used for the estimation of Ribonucleic acid (RNA) and Deoxyribonucleic acid (DNA).

Estimation of DNA

Total DNA in the tissues was estimated by diphenylamine method as described by David Plummer (2001).

**Principle:** Under extreme acid conditions, DNA is initially depurinated quantitatively followed by the dehydration of sugar to hydroxylevulinaldehyde. This aldehyde condenses in acidic medium, with diphenylamine to produce a deep blue coloured condensation product with absorption maximum at 595 nm.

An aliquot of the nucleic acid extract was taken and made upto 2 ml with distilled water and 4 ml of diphenylamine reagent (1 g of diphenylamine dissolved in 100 ml of glacial acetic acid and 2.5 ml of concentrated H₂SO₄ was added and incubated in boiling water bath for 10 min, cooled and read the extinction at 595 nm. Read the test and standard against a water blank. DNA concentration is expressed as μg/ mg tissue.

Estimation of RNA

Total tissue RNA was estimated by Orcinol method as outlined by David Plummer (2001).

**Principle:** The method depends on conversion of the ribose in the presence of hot acid to furfural, which then reacts with orcinol to yield a green colour. The colour formed largely depends on the concentration of HCl, ferric chloride, orcinol, the time of heating at 100°C etc. up to certain maxima.

An aliquot of the nucleic acid extract was taken and made up to 2 ml with distilled water and 3 ml of orcinol reagent (orcinol 2.1 g, FeCl₃-6H₂O 0.1 g were dissolved in 35 ml
absolute alcohol to this 100 ml concentrated HCl was added). The contents were shaken well and incubated in a boiling water bath for 20 minutes, then cooled under running water and the intensity of the colour was read at 665 nm against orcinol blank. Simultaneously standards were run and concentration of RNA was calculated. RNA concentration in tissue is expressed as μg/mg tissue.

**Estimation of Protein**

Protein content of different tissues was quantified by the method of Lowry et al., (1951).

**Principle:** A deep blue color is formed with protein treated with the Folin ciocalteau. Two colour reactions take place simultaneously.

1. The peptide bonds of protein react with copper in alkaline solution.
2. Reduction of phosphomolybdic acid and phosphotungastic acid by the aromatic amino acids tyrosine and tryptophan present in the protein.

The tissue protein was precipitated by the addition of 1 ml of 30 % trichloroacetic acid (TCA) solution followed by centrifugation at 3000 rpm for 30 minutes. It was repeated twice then the precipitate dissolved in 1 ml of 0.1N sodium hydroxide. A known aliquot of this solution was then mixed with 5 ml of alkaline copper reagent (20% sodium carbonate in 0.1N sodium hydroxide containing sodium potassium tartrate and 1% copper sulphate). After 10 minutes 0.5 ml of Folin ciocalteau reagent (FCR) was added and were shaken thoroughly. Then the tubes were kept for color development in dark. For the reference of standard Bovine serum albumin (BSA) was used, the optical density was read at 650 nm. Tissue protein concentration expressed as μg/ mg tissue.

**Estimation of glycogen**

The total glycogen content of tissues were estimated by the method described by Carrol et al., (1956).

**Principle:** The acidic anthrone reagent reacts with hexoses aldopentoses, and hexuronic acids, either free or present in polysaccharides producing a blue-green colour with an absorption maximum at 620 nm. The tissue homogenate was prepared by using 10 ml of 4% TCA and centrifuged at 1500 rpm for 10 minutes. The supernatant is decanted and
precipitate is discarded. To the 2 ml supernatant, 4 ml of anthrone reagent (200 mg of anthrone was dissolved in 100 ml of concentrated sulphuric acid) was added. Then tubes were allowed to cool for 30 minutes. A blank and standards were prepared simultaneously. The optical density was measured at 620 nm.

**Estimation of cholesterol**

Estimation of cholesterol in the tissues was done by Libermann and Burchard’s reaction as described by Abell *et al.*, (1952).

**Principle:** Acetic anhydride reacts with cholesterol in chloroform solution to produce a characteristic blue-green colour.

Tissue of known weight was homogenized in 10 ml of 3:1 alcohol and ether mixture and the homogenate was centrifuged for 10 minutes. The supernatant was collected in a test tube and dried in a water bath. The dried residue was dissolved in 5 ml chloroform, and then 1 ml of acetic anhydride mixture (20 ml of acetic anhydride is mixed with 1 ml of concentrated sulphuric acid) was added and kept in dark room for 15 minutes for color development. Simultaneously blank and standards were run and the optical density was measured at 660 nm. Cholesterol concentration in tissues was expressed as µg/ mg tissue.

**Estimation of sialic acid**

Sialic acid was estimated as per the method developed by Yao *et al.*, (1989) using acidic ninhydrin reaction

**Principle:** By heating a sample solution containing sialoglycoprotein with acidic ninhydrin reagent at 100° C for 10 minutes, a stable color with absorbance maxima at 470 nm was produced. The intensity of the color is proportionate to concentration of sialic acid.

The acid ninhydrin reagent was prepared according to Gaitonde (1967). The reagent contained 250 mg ninhydrin in a mixture of 6 ml of glacial acetic acid and 4 ml of concentrated hydrochloric acid, the content were mixed thoroughly by using a vortex mixture for 30 minutes to get a clear solution. The reagent was prepared fresh just before use. The reaction mixture containing 1 ml of sample solution (the supernatant obtained after homogenization with 5% TCA and centrifugation for 15 minutes at 1500 rpm) to this 1 ml of glacial acetic acid was added followed by 1 ml of acidic ninhydrin reagent. The tubes were covered with aluminum caps or glass beads. The reaction mixture was heated at 100°
C in boiling water bath for exactly 10 minutes and then the mixture was rapidly cooled under tap water. The absorbance was measured at 470 nm. The sialic acid concentration was calculated by running of N-acetyl neuraminic acid. The tissue sialic acid concentration was expressed as µg/ mg tissue.

**Estimation of lactate dehydrogenase (LDH) activity**

LDH activity was estimated by the method described by King (1965).

**Principle:** LDH catalyzes the conversion of pyruvate to lactate and NADH to NAD and vice versa. The LDH activity was directly proportional to the pyruvate formed.

10% tissue homogenate (w/v) was prepared with ice cold 0.8 M sucrose solution. The homogenate was centrifuged at 10,000 rpm at 4°C 1 ml of supernatant was used as enzyme source. To the two sets of test tubes labeled test and control. 1 ml of buffered substrate (2.768 g of lithium lactate dissolved in 200 ml of Glycine NaOH buffer pH 10.4) was added and to the test set 0.1 ml of aliquots of the tissue extract was added to the control tubes 0.1 ml of water was added. And 0.2 ml of NAD⁺ (0.6 M) was added to both sets. The reaction mixture was incubated at 37°C for 15 minutes. The reaction was arrested by the addition of 1 ml of colour reagent, 2,4-dinitrophenyl hydrazine to each tube and the incubation was continued for further 15 minutes, then they were cooled at room temperature. 10 ml of 0.4 N NaOH was added to each tube to make the solution strongly alkaline for the maximal development of the colour of hydrazine favoured. The intensity of the colour was measured at 440 nm exactly two seconds after the addition of alkali to each tube. Blank and standards were run simultaneously. The LDH activity expressed as µM pyruvate formed/ min/ g tissue.

**Estimation of succinic dehydrogenase (SDH) activity**

Succinic dehydrogenase activity was estimated by the method described by Nachlas *et al.*, (1960).

**Principle:** SDH catalyses reduction of iodo-phenyl nitophenyl tetrazolium forming coloured product when extracted with toluene. The intensity of the colour is proportional to the activity of SDH.

10% of tissue homogenate was prepared (w/v) with ice cold 0.8 M sucrose solution. The homogenate was centrifuged at 10,000 rpm for 10 minutes at 4°C. 1 ml supernatant
used as enzyme source for estimation. The reaction mixture contained 0.4 ml of 10 mM sodium succinate, 0.5 ml of 100 mM sodium phosphate buffer (pH 7.4) and 0.5 ml of 2 mM INT: The volume was made up to 2 ml by adding 0.6 ml of distilled water. To this 0.5 ml of enzyme source was added then incubated for 30 minutes at 37°C. 5 ml of glacial acetic acid was added to stop the reaction. The colour was extracted in 5 ml toluene at 10°C. Simultaneously blank and standard were prepared. The optical density was read at 495 nm against toluene blank. The enzyme activity of tissue was expressed asμmoles of formazon formed/ min/ g tissue.

Estimation of 3β-hydroxy steroid dehydrogenase (3βHSD) activity

The activity of 3βHSD is determined by the method described by Shivanandappa and Venkatesh (1997).

Principle: 3βHSD acts upon the oxidation of 3β-hydroxyl group of C\textsubscript{19} and C\textsubscript{21} steroids. The activity was determined by the optical measurement of the rate of conversion of NAD to NADH.

3βHSD standard curve plotting

1 mM solution of NADH was freshly prepared in distilled water. Aliquots of graded concentrations of NADH (0-150 n mol) were allowed to react with 0.5 ml of color reagent (40 mg INT, 10 mg phenazene methosulfate, and 0.5 ml of tween 20 were dissolved in 50 ml distilled water) and colour formed, 2 ml of phthalate buffer (50 mM, pH 3.0 i.e. 2.55 g of potassium hydrogen phthalate dissolved in a mixture of 51 ml of 0.1 N HCl and 2.5 ml Tween 20 was added. pH was adjusted to 3.0 and the volume made upto 250 ml with distilled water) was added to each tube and the absorbance was read at 490 nm against blank. A blank containing all the components except NADH was run simultaneously. A standard curve concentration was prepared by plotting NADH concentration versus absorbance.

Determination of 3βHSD activity in tissues

Tissue homogenate 10% (w/v) are prepared in ice cold tris HCl buffer (0.1 M, pH 7.8) and centrifuged at 12,000 rpm for 5 minutes at 4°C and the supernatant was taken as enzyme source. The activity is determined by the optical measurement of rate of the conversion of NAD to NADH. Two sets of test tubes were taken labeled as test and control.

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The reaction mixture in both sets contains a final volume of 3 ml of 0.1 M tris-HCl buffer pH (7.8) containing NAD (500 μM) and the substrate DHEA or pregnenolone (100 μM) INT (0.8 μM). In the test set the reaction was started by adding the enzyme extract (50 μl) and incubated at 37°C for 60 min. The reaction was stopped by the addition of 2.0 ml of phthalate buffer (pH 3.0). To the control set enzyme extract (50 μl) was added after the addition of phthalate buffer. The turbidity was removed by centrifugation at 3000 rpm for 20 minutes and the supernatant was read at 490 nm using control as reference. The activity of enzyme is expressed as μmoles of NAD converted to NADH/ min/g tissue.

Estimation of 17β-hydroxysteroid dehydrogenase (17βHSD) activity

The activity level of 17β-HSD was measured biochemical according to the method of Jarabak et al., (1962).

Principle: 17βHSD catalyses the reversible reaction of androstenedione into testosterone. NADH acts as co-enzyme for this interconversion. The activity was determined by the optical measurement of the rate of conversion of NADPH to NADP.

Determination of 17β-HSD activity in tissue

The testicular tissue of each animal was homogenized in 15% spectroscopic grade glycerol containing 5 mmol potassium sulphate and 1 mmol EDTA at a tissue concentration of 100 mg/ml (10% w/v). The homogenizing mixture was centrifuged at 10,000 rpm at 4°C. The supernatant (1 ml) was mixed with 1 ml of 440 pmol sodium pyrophosphate buffer (pH 10.2), 40 μl of ethanol containing 0.3 μmol of androstenidione and 960 μl of 25% BSA, making the incubation mixture to a total of 3 ml. The enzyme activity was measured after the addition of 1.1 μmol NADP to the tissue supernatant mixture in a spectrophotometer at 340nm against a blank (without NADP). Simultaneously standards were run and standard curve was prepared by plotting NADP concentration Vs absorbance. The activity levels of the enzyme was calculated from standard curve of NADP and expressed in μmoles of NADPH converted to NADP/min/g tissue.

Estimation of adenosine triphosphatase (ATPases or ATP Phosphonylase activity)

The sodium, potassium, calcium and magnesium dependent ATPases were assayed according to the method described by Jinna et al., (1989).
Principle: Adenosine triphosphatase catalyses the conversion of ATP into ADP. During this conversion phosphorus molecules are liberated.

\[
\text{ATP} \rightarrow \text{ADP} + \text{Pi}
\]

The inorganic phosphorus was assayed according to the method of Fiske and Subbarow (1925). The proteins are precipitated with trichloroacetic acid. The protein free filterate was treated with an acid molybdate sodium which forms phosphomolybdic acid which was reduced by the addition of 1,2,1 amino napthol sulphuric acid reagent to produce blue colour, the intensity of which is proportional to the amount of phosphate present.

The 10% of tissue homogenate (w/v) was prepared by using ice cold tris HCl buffer (0.1 M pH 7.75). The homogenate was centrifuged at 10,000 rpm at 4°C for 15 minutes. The supernatant was taken for assays.

Two sets of test tubes were taken as test and control. To the tubes labeled test and control each having 0.2 ml tris HCl buffer (pH 7.5), 0.1 ml ATP (4.5 mM) was added. 0.1 ml of 100 mM MgCl₂ or 0.1 ml of 100 mM CaCl₂ or 0.1 ml 100 mM NaCl and 100 mM KCl as in the case of specific assays.

To the tubes labeled test 0.1 ml extract added. The reaction mixture was incubated at 37°C for 15 minutes. The reaction was terminated with 2 ml of 3% TCA. To the tubes labeled control 0.1 ml tissue extract was added after adding 2 ml of 3% TCA. The protein was precipitated with TCA by centrifuging the tubes for 5 minutes at 2000 rpm. The inorganic phosphorus present in the supernatant was determined colorimetrically by the method of Fiske and Subbarow (1925).

To the supernatant 1 ml of ammonium molybdate (2.5% molybdate in 1.5N H₂SO₄) and 0.4 ml of ANSA (0.25% Aminonaphthol sulphuric acid containing sodium sulphite 0.3% and sodium metabisulphite 14.63%) reagent was added. Suitable standards were run through each batch of assays. Inclusions of calculated concentration of phosphate solution in the standard made alone for the chromogenicity of phosphate formed in the test. Blank and standards were run simultaneously. The enzymatic activity was expressed in terms of μmoles of inorganic phosphorous formed/ min/ g tissue weight.
Estimation of acid phosphatase (ACP) and alkaline phosphatase (AKP)

The acid phosphatase and alkaline phosphatase activity were estimated by the method of Bergmeyer and Bernt (1963).

**Principle:** The enzyme phosphatase hydrolyzes p-nitrophenyl phosphate. The released p-nitrophenyl is yellow in colour in alkaline medium and is measured at 405 nm. The optimum pHs for acid and alkaline phosphatases are 5.3 and 10.4 respectively.

10% of tissue homogenate (w/v) was prepared with ice-cold 50 mM Citrate buffer (pH 5.3) and the homogenate was centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was used as enzyme source for estimation.

**Determination of acid phosphatase activity**

Two sets of test tubes were taken as test and control. To the test tubes 0.1 ml of enzyme source was added. To both the sets 0.3 ml ACP substrate (EDTA 1.49 g, Citric acid 0.84g, p-nitrophenyl phosphate 0.03 g dissolved in 100 ml distilled water and pH adjusted 5.3), 0.1 ml citrate buffer (pH 5.3) was added and incubated for 15 minute at 37°C. 9.5 ml of 0.085 N NaOH was added. To the control set 0.1 ml of enzyme source was added after the addition of 0.085 N NaOH. Simultaneously blank and standards were prepared. The colour formed was read at 405 nm using control as reference. ACP activity was expressed as pmoles of p-nitrophenyl formed/ min/ g tissue.

**Determination of alkaline phosphatase activity**

Two sets of test tubes were taken as test and control. To the test tubes 0.1 ml of enzyme source was added. To both the sets 0.3 ml AKP substrate (Glycine 375 mg, MgCl₂ 10 mg, p-nitrophosphyl-phosphate 165 mg were dissolved in 42 ml of 0.1 N NaOH and diluted to 100 ml with distilled water. Adjust pH to 10.5). 0.1 ml Glycine NaOH buffer (pH 10.4) was added and incubated for 15 minutes at 37°C. Add 9.5 ml of 0.085 N NaOH. To the control set 0.1 ml of enzyme source was added after the addition of 0.085 N NaOH. Simultaneously blank and standards were prepared. The colour formed was read at 405 nm using control as reference. Tissue AKP is expressed as μmoles p-nitrophenyl formed/ min/ g tissue.
Preparation of tissue homogenate

The tissues were thawed and homogenized in 10% w/v ice-cold 0.05 M potassium phosphate buffer (pH 7.4). 0.2 ml of the homogenate was used for TBARS estimation and 1.0 ml of the homogenate was mixed with 10% trichloroacetic acid (TCA) and centrifuged for tissue GSH estimation. The remaining homogenate was centrifuged at 40,000 x g for 60 min and the supernatant was used for estimations of superoxide dismutase (SOD) and catalase (CAT). Protein concentration was estimated according to Bradford 1976.

Reduced glutathione (GSH)

GSH level was measured following the method of Ellman (1959), modified by Hissin and Hilf (1973). The homogenate (720 µl) was double diluted and 5% TCA was added to it to precipitate the protein content of the homogenate. After centrifugation (10,000 x g for 5 minutes) the supernatant was taken, 5,5'-dithiolbis-2-nitrobenzoic acid (DTNB) solution (Ellman’s reagent) was added to it and the absorbance was measured at 412 nm. A standard graph was drawn using different concentrations of standard GSH solution (1 mg/ml). With the help of the standard graph, GSH contents in the liver homogenates of the experimental animals were calculated. 13.6 x 10³ M⁻¹ cm⁻¹.

Ascorbic acid

The ascorbic acid level was estimated by the 2, 4-dinitrophenyl-hydrazine method by Roe and Kuether (1942) standardized by Kaplan and Pesce (1987). Homogenate of 0.6 ml was added to 2 ml of freshly prepared metaphosphoric acid, mixed well in a vortex mixer and centrifuged for 10 min at 2500 rpm. 1.2 ml of the clear supernatant was taken. In seven other tubes 0.1, 0.2, 0.4, 0.6, 0.8, 1 and 1.2 ml, respectively, of working standards were taken and the volume made up to 1.2 ml with metaphosphoric acid. To the blank 1.2 ml of metaphosphoric acid was added. 0.4 ml of dinitrophenyl-hydrazine-thiourea-copper sulphate was added to all the tests, mixed thoroughly and the tubes were incubated in a water bath at 37°C for 3 h with glucuronic acid and ascorbate-2-sulphate. The tubes were removed from the water bath and chilled for 10 min in an ice bath. 2 ml of chilled sulphuric acid (12 mol/l) was added to all the tubes and mixed well in a vortex mixer at room temperature. The absorbance was read at 520 nm against the reagent blank to express the result in µmol of ascorbic acid / gm wet tissue.
Thiobarbaturic acid reactive substances (TBARS)

The product of the reaction between malondialdehyde (MDA) and thiobarbituric acid reactive substances Okhawa et al., (TBARS) were measured by a modified method of Esterbauer and Cheesman, (1990). For each sample to be assayed, four tubes were set up containing 100, 150, 200 and 250 µL of tissue homogenate, 100 µL of 8.1% SDS, 750 µL of 20% acetic acid, and 750 µL of 0.8% aqueous solution of TBA. The volume was made up to 4 mL with distilled water, mixed thoroughly and heated at 95°C for 60 minutes. After cooling, 4 mL of n-butanol was added to each tube, the contents mixed thoroughly, and then centrifuged at 3000 rpm for 10 minutes. The absorption of the clear upper (n-butanol) layer was measured using a Shimadzu UV-1601 (Japan) spectrophotometer at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex 1.56 x 105 cm-1 M-1 and was expressed in µmol TBARS/mg tissue protein. (ε= 1.56 X10² mMT'cm'1)

Protein carbonylation assay

This assay measures protein carbonyls, an indicator of protein oxidation, using 2, 4-dinitrophenylhydrazine (DNPH).17 DNPH reacts with protein carbonyls to form hydrazones that can be measured spectrophotometrically. Briefly, 800 µL of 2.5 mM DNPH was dissolved in HCl and mixed with 200 µL of protein sample (1 mg). Equal amounts of protein samples without DNPH were used as controls. Control and DNPH-treated samples were then incubated in the dark for one hour and vortexed every 15 min. After the incubation, 1 mL of a 20% trichloroacetic acid (TCA) solution was added to each tube and the tubes were placed on ice for 5 min after vortexing. The tubes were then centrifuged at 10 000× g for 10 min at 4 °C. The supernatant was discarded, and the pellet was resuspended in 1 mL of 10% TCA solution, and then ethanol/ethyl acetate mixture (1:1) was then added. This procedure was repeated twice, and the pellet was resuspended in 500 µL of guanidine hydrochloride. After centrifugation, the supernatant from the control and treated tubes were then the absorbance was determined at a wavelength of 385 nm. 22,000 M⁻¹ cm⁻¹.

Superoxide dismutase activity (SOD)

Liver SOD activity was assayed by the method of Kakkar et al., (1984). Reaction mixture contained 1.2 ml of sodium pyrophosphate buffer (0.052 mM, pH 7.0), 0.1 ml of phenazine
methosulphate (PMS) (186 μM), 0.3 ml of nitro blue tetrazolium (NBT) (300 μM). 0.2 ml of the supernatant obtained after centrifugation (1500 x g, 10 min followed by 10,000 x g, 15 min) of 10% liver homogenate was added to reaction mixture. Enzyme reaction was initiated by adding 0.2 ml of NADH (780 μM) and stopped precisely after 1 min by adding 1 ml of glacial acetic acid. Amount of chromogen formed was measured by recording color intensity at 560 nm. Results are expressed as units/mg protein.

**Catalase**

Supernatant (50 μl) was added to a 3.0 ml cuvette that contained 1.95 ml of 50 mM phosphate buffer (pH 7.0). 1.0 ml of 30 mM hydrogen peroxide was added and changes in absorbance were followed for 30 sec at 240 nm at 15-sec intervals. Catalase activity was expressed as IU per mg protein (Aebi, 1974).

**Glutathione-s-transferase (GST)**

GST catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. GST activity was measured by the method of Habig and Jakoby (1974). The reaction mixture contained suitable amount of the enzyme (25 μg of protein in homogenates), 1 ml of KH₂PO₄ buffer, 0.2 ml of EDTA, 0.1 ml of 1-chloro-2,4-dinitrobenzene (CDNB), and GSH. The reaction was carried out at 37°C and monitored spectrophotometrically by the increase in absorbance of the conjugate of GSH and CDNB at 340 nm. A blank was run in absence of the enzyme. One unit of GST activity is 1 μmol product formation per minute (9.6 x 10⁷ M⁻¹ cm⁻¹). 

**Analysis of DNA in agarose gel**

Tissue (200 mg) were lysed at 37°C for 60 min in a buffer containing 0.5% Triton X-100, 5 mM Tris buffer (pH 7.4), and 20 mM EDTA. The lysate was then incubated in proteinase K (100, ug/ml) for 2 h at 37°C. After RNase treatment for 1 h at 37°C, DNA was extracted with an equal volume of phenol/ chloroform, 1:1, and precipitated with 1/10th vol 7 M ammonium acetate and 2.5 vol ice-cold ethanol at room temperature for 1 h. DNA samples were loaded on to 1.8 % agarose gels and run at 90 V for 2 h (Wyllie et al., 1980).
Assay of serum testosterone concentrations

The levels of serum testosterone were measured using the testosterone kit from Monobind Ind., Lakeforest, USA, following the immunoenzymatic method in ELISA reader (ELICO) and according to the standard protocol given by National Institute of Health and Family Welfare (NIHFW, New Delhi) (Srivastava, 2002). Horseradish peroxidase was used as an enzyme-labeled antigen that made a completion with unlabelled antigen for binding with a limited number of antibody sites on the micro plates (solid phase). In each sample, testosterone concentration was calculated from a standard curve with 5 standards. The absorbance of standard and sample was monitored against the blank at 450 nm. The cross-reaction of the testosterone antibody to dehydrotestosterone was 10.0% and intra-assay run precision had a co-efficient of variation of 6.2%. There was no inter-assay variation as all samples were assayed at a time. Results of testosterone are expressed as ng/ml serum.

Statistical analysis

The statistical analysis has been performed according to routine formulae found in standard work on biological statistics (Fisch, 1936; Snedecor, 1946; Wilks, 1949; Robert et al., 1980).

Following abbreviations and formulae are used.

\[ X \quad \text{independent variable} \]
\[ n \quad \text{number of observations} \]
\[ df \quad \text{degree of freedom} \]

Whenever the numerical data provided, it is expressed as \((X \pm SE)\). The standard deviation and standard error of the mean were calculated by using the following formulae.

1. Standard deviation
   \[ S = \sqrt{\frac{(X_1 - X_2)^2}{n-1}} \]
2. Standard error
   \[ SE = \frac{S}{\sqrt{n}} \quad \text{where } S \text{ is Standard deviation} \]
3. Degree of freedom for a difference of two means \(n_1\) and \(n_2\) variable
   \[ df = n_1 + n_2 - 2 \]
4. Statistical significant \((P \leq 0.05)\) among the various parameters assessed was established by using ANOVA and Dunnet’s test (1955).
OBSERVATIONS

Experiment I

Effect on testes and accessory reproductive organs, spermatogenic cells, biochemical contents, dehydrogenase, phosphatase enzymes activity and oxidative stress parameters in the testes and epididymides in the mice on exposure to methomyl.

Testes and accessory reproductive organs weight (Table 1.1; Graph 1.1)

Testes

The mean weight of the testes in control mice was 745.6 mg. The mean weights of the testes with 1, 2, 3 and 4 mg methomyl treatments were 742.8, 700.5, 659.7 and 630.5 mg respectively. There was a significant decrease in the testes weight with 2, 3 and 4 mg methomyl treatments. However, 1 mg methomyl treatments showed no significant change in the testes weight when compared with that of the control mice.

Epididymides

The mean weight of the epididymides in control mice was 333.4 mg. The mean weight of the epididymides with 1, 2, 3 and 4 mg methomyl treatments was 331.6, 327.5, 317.5 and 299.3 mg respectively. There was a significant decrease in the epididymides weight with 2, 3 and 4 mg methomyl treatments. However, treatment with 1 mg methomyl caused no significant change in the epididymides weight when compared with that of the control mice.

Vasa deferentia

The mean weight of the vasa deferentia in control mice was 190.9 mg. The mean weights of the vasa deferentia with 1, 2, 3 and 4 mg methomyl treatments were 189.5, 188.8, 162.0 and 158.6 mg respectively. There was a significant decrease in the weight of vasa deferentia with 2, 3 and 4 mg methomyl treatments. However, 1 mg methomyl treatment showed no significant change in the vasa deferentia weight when compared with that of the control mice.
Seminal vesicles

The mean weight of the seminal vesicles in control mice was 803.0 mg. The mean weights of seminal vesicles with 1, 2, 3 and 4 mg methomyl were 802.6, 801.9, 750.5 and 710.5 mg respectively. There was a significant decrease in the weight of the seminal vesicles with 3 and 4 mg methomyl treatments. However, there was no significant change in the weight of the seminal vesicles with 1 and 2 mg methomyl treatment when compared with that of the control mice.

Prostate gland

The mean weight of the prostate gland in control mice was 95.6 mg. The mean weights of the prostate gland with 1, 2, 3 and 4 mg methomyl were 94.9, 94.0, 83.9 and 77.5 mg respectively. There was a significant decrease in the prostate gland weight with 3 and 4 mg methomyl treatment. However, there was no significant change in the weight of the prostate gland with 1 and 2 mg methomyl treatments when compared with that of control mice.

Coagulatory glands

The mean weight of the coagulatory glands in control mice was 175.4 mg. The mean weights of the coagulatory glands with 1, 2, 3 and 4 mg methomyl were 172.8, 171.1, 161.3 and 154.6 mg respectively. There was a significant decrease in the coagulatory glands weight with 3 and 4 mg methomyl treatments. However, there was no significant change in the weight of the coagulatory glands with 1 and 2 mg methomyl treatment when compared with that of the control mice.

Cowper’s glands

The mean weight of the Cowper’s glands in control mice was 155.0 mg. The mean weights of the Cowper’s glands with 1, 2, 3 and 4 mg methomyl were 153.7, 153.0, 145.9 and 139.0 mg respectively. There was a significant decrease in the Cowper’s glands weight with 2, 3 and 4 mg methomyl treatments. However, there was no significant change in the weight of Cowper’s glands with 1 mg methomyl treatments when compared with that of the control mice.

Study on testes and accessory reproductive organs weight revealed that treatment with 3 and 4 mg methomyl caused significant decrease in the weight of the testes and
accessory reproductive organs (epididymides, vasa deferentia, seminal vesicles, prostate gland, coagulatory glands and Cowper's glands). However, treatment with 1 and 2 mg methomyl treatment caused no significant change in the weight of the testes and accessory reproductive organs, except seminal vesicles, prostate gland and coagulatory glands weight where there was a significant decrease with 2 mg methomyl treatment when compared with those of the corresponding parameters of the control mice (Table 1.1; Graph 1.1).

**Number and diameter of spermatogenic and Leydig cells**  
(Table 1.2; Graphs 1.2 and 1.3)

In the control mice the mean numbers of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 67.9, 92.1, 100.6, 168.1 and 44.4 respectively and the mean diameter of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 8.0, 9.3, 7.7, 6.3 and 9.6 μm respectively.

In the mice treated with 1 mg methomyl, the mean numbers of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 67.2, 91.6, 99.7, 167.1 and 43.4 respectively and the mean diameter of spermatogonia, primary and secondary spermatocytes, spermatids and Leydig cells were 7.6, 9.0, 7.4, 6.1 and 9.3 μm respectively. There was no significant change in the number and diameter of spermatogenic and Leydig cells when compared with those of the corresponding parameters of the control mice.

In the mice treated with 2 mg methomyl, the mean numbers of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 64.1, 91.1, 97.2, 164.7 and 42.6 respectively and the mean diameter of spermatogonia, primary and secondary spermatocytes, spermatids and Leydig cells were 7.3, 8.4, 7.1, 5.8 and 8.7 μm respectively. There was no significant change in the number of primary spermatocytes, Leydig cells and diameter of spermatogonia when compared with those of the corresponding parameters of the control mice.

In the mice treated with 3 mg methomyl, the mean numbers of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 62.2, 87.6, 94.6, 162.9 and 38.9 respectively and the mean diameter of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 6.4, 7.3, 6.7, 5.0 and 7.5 μm respectively. There was a significant decrease in the number and diameter of
spermatogenic and Leydig cells when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl the mean numbers of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 59.4, 85.6, 92.4, 161.3 and 38.3 respectively and the mean diameters of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 6.1, 7.1, 6.3, 4.9 and 6.4 μm respectively. There was a significant decrease in the number and diameter of spermatogenic and Leydig cells when compared with those of the corresponding parameters of the control mice.

The findings of the present study on number and diameter of spermatogenic cells (spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells revealed that there was a significant decrease in the number and diameter of spermatogenic and Leydig cells with 2, 3 and 4 mg methomyl treatments except with 2 mg there was no significant change in the number of primary spermatocytes, Leydig cells and diameter of spermatogonia. However, treatment with 1 mg methomyl showed no significant change in the number and diameter of spermatogenic and Leydig cells when compared with those of the corresponding parameters of the control mice (Table 1.2; Graphs 1.2 and 2.3).

**Testis histology**

Histologic observations of the testis of the control mouse revealed that the seminiferous tubules contain all stages of spermatogenesis and interstitial cells. The different stages of spermatogenesis are spermatogonia attached on the basement membrane of seminiferous tubule and towards the lumen the primary spermatocytes, secondary spermatocytes and spermatids were found. Lumen of the seminiferous tubules filled with sperms. Interstitial tissue showed clusters of Leydig cells (Fig. 1).

Histologic observations of the testis of the mouse treated with 1 mg methomyl revealed decrease in the number of spermatocytes, spermatids and sperms in the lumen of seminiferous tubules and interstitial tissue contains clusters of Leydig cells (Fig. 2). Histologic study of the testis of the mouse treated with 2 mg methomyl exhibited decrease in the number of spermatogenic cells, formation of giant cells, vacuoles and loss of number of sperms in the lumen of the seminiferous tubules with disturbed architecture of Leydig cells (Fig. 3). Histologic examination of the testis of the mouse treated with 3 mg methomyl
showed formation of giant cells, decreased number of spermatogenic cells and lumen with decreased number of sperms. Leydig cells are in deformed condition (Fig. 4). Histologic observations of the testis of the mouse treated with 4 mg methomyl revealed formation of giant cells, vacuoles and marked reduction in spermatogenic cells. Seminiferous tubules are atrophied. Lumen showed tissue debris and clumped sperms. Leydig cells are in deformed condition (Fig. 5).

**Biochemical contents of the testis (Table 1.3; Graph 1.4)**

In the control mice the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.51, 4.68, 147.7, 6.52, 9.25 and 15.6 µg respectively in the testis. In the mice treated with 1 mg methomyl, the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.46, 4.62, 147.1, 6.48, 9.3 and 15.1 µg respectively. There was no significant change in the level of biochemical contents of the testis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 2 mg methomyl the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.44, 4.58, 146.4, 6.43, 9.42 and 14.8 µg respectively in the testis. There was a significant decrease in the levels of biochemical contents protein and glycogen except cholesterol where it was increased significantly. However, the level of DNA and RNA not changed significantly when compared with those of the corresponding parameters of the control mice.

In the mice treated with 3 mg methomyl the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.33, 4.13, 140.5, 6.15, 9.62 and 13.6 µg respectively in the testis. There was a significant decrease in the level of biochemical contents, except cholesterol where it was increased significantly in the testis. However, DNA and RNA not changed significantly when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.11, 3.87, 135.5, 5.53, 9.81 and 12.5 µg respectively in the testis. There was a significant decrease in the level of biochemical contents, except cholesterol where it was increased significantly when compared with those of the corresponding parameters of the control mice.
The findings of the present study on biochemical contents of the testis revealed that, in the mice treated with 2, 3 and 4 mg methomyl caused significant decrease in the level of DNA, RNA, protein, glycogen and sialic acid, whereas cholesterol content was increased significantly. However, the level of DNA was not changed significantly in 2 mg methomyl treated mice. In the mice treated with 1 mg methomyl, there was no significant change in the level of the biochemical contents of the testis when compared with those of the corresponding parameters of the control mice (Table 1.3; Graph 1.4).

**Testis dehydrogenase and phosphatase enzymes activity (Table 1.4; Graph 1.5)**

In the control mice, the activity of LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 9.06, 12.20, 0.44, 0.94, 7.45, 9.00, 6.10, 18.80 and 14.10 µmoles respectively in the testis. In the mice treated with 1 mg methomyl the activity of LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 9.10, 12.15, 0.43, 0.94, 7.42, 8.96, 6.08, 18.60 and 14.05 µmoles respectively. There was no significant change in the activity of enzymes in the testis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 2 mg methomyl, the activity of LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 9.11, 12.09, 0.41, 0.85, 7.38, 8.90, 6.07, 18.50 and 14.00 µmoles respectively in the testis. There was no significant change in SDH, LDH, 3βHSD, Na⁺-K⁺ATPase, Ca⁺⁺ATPase, Mg⁺⁺ATPase and ACP activity whereas AKP activity increased significantly when compared with those of the corresponding parameters of the control mice.

In the mice treated with 3 mg methomyl, the activity of LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 10.08, 11.60, 0.37, 0.79, 7.00, 7.80, 6.05, 17.60 and 14.90 µmoles respectively in the testis. There was a significant decrease in the activity of SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase and ACP, whereas the activity of the LDH and AKP were increased significantly when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl, the activity of LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 10.30, 11.15, 0.32, 0.72, 6.42, 7.10, 5.42, 16.20 and 15.50 µmoles respectively in the testis. There was a significant decrease in the activity of SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase,
Ca\(^{2+}\)ATPase and ACP. However, the activity of LDH and AKP were increased significantly when compared with those of the corresponding parameters of the control mice.

Study on the activity of dehydrogenase, phosphatase and steroidogenic enzymes in the testis revealed that, in the mice treated with 3 and 4 mg methomyl showed significant decrease in the activity of SDH, 3\(\beta\)HSD, 17\(\beta\)HSD, Na\(^{+}\)-K\(^{+}\)ATPase, Mg\(^{2+}\)ATPase, Ca\(^{2+}\)ATPase and ACP. However, the activity of the LDH and AKP were increased significantly, except Ca\(^{2+}\)ATPase was not changed significantly in 3 mg treated mice. In the mice treated with 1 and 2 mg methomyl caused no significant change in the activity of enzymes, except in 2 mg methomyl treated mice where the activity of the 17\(\beta\)HSD decreased significantly when compared with those of the corresponding parameters of the control mice (Table 1.4; Graph 1.5).

**Antioxidants and oxidative stress byproducts of the testis in the mice on exposure to methomyl (Table 1.5; Graph 1.6)**

In the control mice the level of GSH, ascorbic acid, TBARS and protein carbonyl was 9.75, 362, 0.24 and 1.24 \(\mu\)g respectively in the testis. In the mice treated with 1 mg methomyl, the level of GSH, ascorbic acid, TBARS and protein carbonyl was 9.72, 360, 0.26 and 1.26 \(\mu\)g respectively. There was no significant change in the level of antioxidants and oxidative stress byproducts contents of the testis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 2 mg methomyl the level of GSH, ascorbic acid, TBARS and protein carbonyl was 9.61, 356, 0.28 and 1.31 \(\mu\)g respectively in the testis. There was a no significant decrease in the levels of antioxidants and oxidative stress byproducts contents, except the level of GSH where it was decreased significantly when compared with those of the corresponding parameters of the control mice.

In the mice treated with 3 mg methomyl the level of GSH, ascorbic acid, TBARS and protein carbonyl was 9.58, 329, 0.36 and 1.45 \(\mu\)g respectively in the testis. There was significant decrease in the level of GSH and ascorbic acid contents, whereas TBARS and Protein carbonyl where it was increased significantly when compared with those of the corresponding parameters of the control mice.
In the mice treated with 4 mg methomyl the level of GSH, ascorbic acid, TBARS and protein carbonyl was 8.72, 308, 0.44 and 1.52 μg respectively in the testis. There was a significant decrease in the level of GSH and ascorbic acid contents, whereas TBARS and Protein carbonyl where it was increased significantly when compared with those of the corresponding parameters of the control mice.

The findings of the present study on biochemical contents of the testis revealed that, in the mice treated with 3 and 4 mg methomyl caused significant decrease in the level of GSH and ascorbic acid contents, whereas TBARS and protein carbonyl content was increased significantly. In the mice treated with 1 and 2 mg methomyl, there was no significant change in the levels of the antioxidants and oxidative stress byproducts of testis, except the level of GSH was decreased significantly in 2 mg methomyl treated mice when compared with those of the corresponding parameters of the control mice (Table 1.5; Graph 1.6).

Oxidative stress enzymes SOD, CAT and GST of the testis in the mice on exposure to methomyl (Table 1.5; Graph 1.6)

In the control mice, the activity of SOD, CAT and GST was 42.8, 151 and 4.2 μmoles respectively in the testis. In the mice treated with 1 mg methomyl the activity of SOD, CAT and GST was 42.0, 148 and 4.0 μmoles respectively. There was no significant change in the activity of enzymes in the testis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 2 mg methomyl, the activity of SOD, CAT and GST was 38.9, 146 and 3.5 μmoles respectively in the testis. There was no significant change in the activity of CAT and SOD. However, activity of GST was decreased significantly when compared with that of the control mice.

In the mice treated with 3 mg methomyl, the activity of SOD, CAT and GST was 33.4, 133 and 3.2 μmoles respectively in the testis. There was a significant decrease in the activity of CAT, SOD and GST, when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl, the activity of SOD, CAT and GST was 30.1, 122 and 2.6 μmoles respectively in the testis. There was a significant decrease in the
activity of SOD, CAT and GST when compared with those of the corresponding parameters of the control mice.

Study on the activity of oxidative stress enzymes in the testis revealed that, in the mice treated with 3 and 4 mg methomyl showed significant decrease in the activity of CAT, SOD and GST. However, in the mice treated with 1 and 2 mg methomyl caused no significant change in the activity of enzymes, except the activity of GST was decreased significantly in 2 mg treated mice when compared with those of the corresponding parameters of the control mice (Table 1.5; Graph 1.6).

Biochemical contents of the epididymis (Table 1.6; Graph 1.7)

In the control mice the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.40, 4.52, 220.20, 7.40, 8.40 and 12.20 µg respectively in the epididymis. In the mice treated with 1 mg methomyl, the level of DNA, RNA protein, glycogen, cholesterol and sialic acid was 2.38, 4.50, 216.20, 7.36, 8.45 and 12.14 µg respectively in the epididymis. There was no significant change in the level of biochemical contents of the epididymis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 2 mg methomyl the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.35, 4.40, 210.3, 7.30, 8.52 and 12.10 µg respectively in the epididymis. There was no significant change in the level of biochemical contents in the epididymis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 3 mg methomyl the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.34, 4.10, 198.4, 6.64, 9.68 and 11.40 µg respectively in the epididymis. There was a significant decrease in the level of biochemical contents, except cholesterol content where it was increased significantly in the epididymis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.10, 4.00, 190.1, 6.60, 9.90 and 11.12 µg respectively in the epididymis. There was a significant decrease in the level of biochemical contents, except cholesterol content where it was increased significantly in the epididymis when compared with those of the corresponding parameters of the control mice.
The findings of the present study on biochemical contents of the epididymis revealed that in the mice treated with 3 and 4 mg methomyl caused significant decrease in the level of DNA, RNA, protein, glycogen and sialic acid contents, whereas cholesterol level was increased significantly. However, treatment with 1 and 2 mg methomyl showed no significant change in the level of biochemical contents when compared with those of the corresponding parameters of the control mice (Table 1.6; Graph 1.7).

**Epididymis dehydrogenase and phosphatase enzymes activity (Table 1.7; Graph 1.8)**

In the control mice the activity of LDH, SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 15.20, 14.12, 8.62, 10.70, 6.70, 15.40 and 13.60 μmoles respectively in epididymis. In the mice treated with 1 mg methomyl the activity of LDH, SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 15.16, 14.08, 8.55, 10.62, 6.65, 15.30 and 13.66 μmoles respectively in the epididymis. There was no significant change in the activity of the enzymes in the epididymis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 2 mg methomyl the activity of LDH, SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 15.12, 13.98, 8.45, 10.54, 6.62, 15.26 and 13.94 μmoles respectively in the epididymis. There was no significant change in the activity of the enzymes when compared with those of the corresponding parameters of the control mice.

In the mice treated with 3 mg methomyl the activity of LDH, SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 16.00, 13.10, 7.56, 9.76, 6.56, 14.65 and 14.80 μmoles respectively in the epididymis. The activity of SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase and ACP were decreased significantly, whereas the activity of the LDH and AKP were increased significantly in the epididymis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl the activity of LDH, SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 16.70, 12.84, 7.10, 9.51, 5.96, 14.32 and 15.36 μmoles respectively in the epididymis. There was a significant decrease in the activity of SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase and ACP in the epididymis. However,
the activity of the LDH and AKP were increased significantly in the epididymis when compared with those of the corresponding parameters of the control mice.

The findings of the present results suggest that the treatment with 3 and 4 mg methomyl, caused significant decrease in the activity of SDH, Na+-K+ATPase, Mg++ATPase, Ca++ATPase and ACP in the epididymis, whereas the activity of LDH and AKP were increased significantly. The mice treated with 1 and 2 mg methomyl caused no significant change in the enzymes activity of epididymis when compared with those of the corresponding parameters of the control mice (Table 1.7 Graph 1.8).

Serum Testosterone levels (Table 1.8; Graph 1.9)

The level of serum testosterone was 52.40 ng/ ml in the control mice. The mean serum testosterone level in 1, 2, 3 and 4 mg/kg methomyl treatment was 51.30, 48.64, 32.78 and 20.62 ng/ ml respectively. There was a significant decrease in the levels of serum testosterone in 3 and 4 mg methomyl treatments. However, treatment with 1 and 2 mg methomyl showed no significant change in the level of serum testosterone when compared with that of the control mice (Table 1.8; Graph 1.9).
Experiment II

Temporal effect on testes and accessory reproductive organs, spermatogenic cells, biochemical contents, dehydrogenase, phosphatase enzymes activity and oxidative stress parameters in the testes and epididymis in the mice on exposure to methomyl.

Testes and accessory reproductive organs weight (Table 1.9; Graph 1.10)

Testes

The mean weight of the testes in control mice was 745.6 mg. The mean weights of the testes with 4 mg methomyl treatment for 5, 10, 20 and 30 days were 743.7, 700.9, 659.7 and 630.5 mg respectively. Methomyl treatment for 10, 20 and 30 days caused significant decrease in the testes weight. However, methomyl treatment for 5 days caused no significant change in the testes weight when compared with that of control mice.

Epididymides

The mean weight of the epididymides in control mice was 333.4 mg. The mean weight of the epididymides with 4 mg methomyl treatment for 5, 10, 20 and 30 days was 338.0, 324.2, 308.5 and 299.3 mg respectively. In the mice treated with methomyl for 10, 20 and 30 days, epididymides weight was decreased significantly. Treatment with methomyl for 5 days caused no significant change in the weight of the epididymides when compared with that of control mice.

Vasa deferentia

The mean weight of the vasa deferentia in control mice was 190.9 mg. The mean weights of the vasa deferentia with 4 mg methomyl treatment for 5, 10, 20 and 30 days was 189.8, 180.9, 162.3 and 158.6 mg respectively. Treatment with methomyl for 10, 20 and 30 days caused significant decrease in the vasa deferentia weights. However, in the mice treated with methomyl for 5 days the weight of the vasa deferentia was not changed significantly when compared with that of control mice.

Seminal vesicles

The mean weight of the seminal vesicles in control mice was 803.0 mg. The mean weights of the seminal vesicles with 4 mg methomyl treatment for 5, 10, 20 and 30 days was 802.0, 801.7, 751.8 and 710.5 mg respectively. There was a significant decrease in the
weights of seminal vesicles in the mice treated with methomyl for 20 and 30 days. However, 5 and 10 days methomyl treatment caused no significant change in the weight of the seminal vesicles when compared with that of the control mice.

**Prostate gland**

The mean weight of the prostate gland in control mice was 95.6 mg. The mean weights of the prostate gland with 4 mg methomyl treatment for 5, 10, 20 and 30 days was 95.0, 94.2, 85.2 and 77.5 mg respectively. There was a significant decrease in the prostate gland weight in mice treated with methomyl for 10, 20 and 30 days. However, 5 days methomyl treatment caused no significant change in the weight of the prostate gland when compared with that of the control mice.

**Coagulatory glands**

The mean weight of the coagulatory glands in control mice was 175.4 mg. The mean weight of the coagulatory glands with 4 mg methomyl treatment for 5, 10, 20 and 30 days was 173.0, 169.8, 161.7 and 154.6 mg respectively. There was a significant decrease in the coagulatory glands weight in mice treated with methomyl for 10, 20 and 30 days. However, the weights of the coagulatory glands were not changed significantly with methomyl treatment for 5 days when compared with that of the control mice.

**Cowper's glands**

The mean weight of the Cowper's glands in control mice was 155.0 mg. The mean weight of Cowper's glands with 4 mg methomyl treatment for 5, 10, 20 and 30 days was 154.0, 150.3, 146.3 and 139.0 mg respectively. There was a significant decrease in the weights of the Cowper's glands with methomyl treatment for 10, 20 and 30 days. However, weight of Cowper's glands was not changed significantly with methomyl exposure for 5 days when compared with that of the control mice.

Results of the present study on testes and accessory reproductive organs weight revealed that in the mice treated with 4 mg methomyl for 10, 20 and 30 days caused significant decrease in the weight of testes, epididymides, vasa deferentia, seminal vesicles, prostate gland, coagulatory glands and Cowper's glands, except seminal vesicles weight was not decreased significantly in the mice exposed to methomyl for 10 days. However, methomyl exposure for 5 days caused no significant change in the weight of the testes and
Accessory organs when compared with those of the corresponding parameters of the control mice (Table 1.9; Graph 1.10).

**Number and diameter of spermatogenic and Leydig cells**
*(Table 1.10; Graph 1.11 and 1.12)*

In the control mice, the mean number of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 67.9, 92.1, 100.6, 168.1 and 44.4 respectively and the mean diameter of the spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells was 8.8, 9.3, 7.7, 6.3 and 9.6 μm respectively.

In the mice treated with 4 mg methomyl for 5 days, the mean number of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells was 67.3, 91.7, 99.9, 167.4 and 43.8 respectively and the mean diameter of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells was 7.4, 9.0, 7.3, 6.0 and 9.1 μm respectively. There was no significant change in the number and diameter of spermatogenic and Leydig cells when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 10 days, the mean numbers of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells was 66.5, 89.2, 97.0, 165.0 and 42.9 respectively and the mean diameters of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells was 6.6, 8.8, 6.8, 5.4 and 8.5 μm respectively. There was a significant decrease in the number and diameter of spermatogenic and Leydig cells, except the number of Leydig cell and diameter of primary spermatocytes was not changed significantly when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 20 days, the mean number of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells was 62.4, 86.9, 94.3, 163.2 and 39.2 respectively and the mean diameter of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells was 6.3, 7.6, 6.7, 5.1 and 7.3 μm respectively. There was a significant decrease in the number and diameter of spermatogenic and Leydig cells when compared with those of the corresponding parameters of the control mice.
In the mice treated with 4 mg methomyl for 30 days, the mean number of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells was 59.4, 85.6, 92.4, 161.3 and 38.3 respectively and the mean diameter of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells was 6.1, 7.1, 6.3, 4.9 and 6.4 μm respectively. There was a significant decrease in the number and diameter of spermatogenic and Leydig cells, when compared with those of the corresponding parameters of the control mice.

The findings of the present study on number and diameter of spermatogenic and Leydig cells revealed that the treatment of mice with 4 mg methomyl for 10, 20 and 30 days caused significant decrease in the number and diameter of spermatogenic cells (spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids) and Leydig cells, except the number of Leydig cell and diameter of primary spermatocytes was not changed significantly in the mice treated with 4 mg methomyl for 10 days, whereas treatment with 4 mg methomyl for 5 days caused no significant change in the number and diameter of spermatogenic and Leydig cells when compared with those of the corresponding parameters of the control mice (Table 1.10; Graph 1.11 and 1.12).

**Testis histology**

Histologic observations of the testis of the control mouse revealed that the seminiferous tubules contain all stages of spermatogenesis and interstitial cells. The different stages of spermatogenesis are spermatogonia attached on the basement membrane of seminiferous tubule and towards the lumen the primary spermatocytes, secondary spermatocytes and spermatids were found. Lumen of the seminiferous tubules filled with sperms. Interstitial tissue showed clusters of Leydig cells (Fig. 6).

Histologic observations of the testis of the mouse treated with 4 mg methomyl for 5 days showed normal spermatogenesis. The seminiferous tubules are closely packed, there was decreased number of spermatocytes, spermatids and sperms in the lumen. The tubular spaces are packed with interstitial tissue (Fig. 7). Histologic study of the testis of the mouse treated with 4 mg methomyl for 10 days showed seminiferous tubules with disturbed spermatogenesis. Lumen of the seminiferous tubules exhibits loss of sperms. Leydig cells are in deformed condition (Fig. 8). Histologic examination of the testis of the mouse treated with 4 mg methomyl for 20 days showed formation of giant cells, decreased number of spermatogenic cells and lumen with loss of sperms. Seminiferous tubules are atrophied.
Leydig cells are in deformed condition (Fig. 9). Histologic observations of the testis of the mouse treated with 4 mg methomyl for 30 days revealed formation of giant cells, vacuoles and marked reduction in spermatogenic cells. Seminiferous tubules are atrophied. Lumen showed tissue debris and clumped sperms. Leydig cells are in deformed condition (Fig. 10).

**Biochemical contents of the testis (Table 1.11; Graph 1.13)**

In the control mice the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.51, 4.68, 147.7, 6.52, 9.25 and 15.6 μg respectively in the testis. In the mice treated with 4 mg methomyl for 5 days, the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.48, 4.64, 147.3, 6.45, 9.33 and 15.0 μg respectively in the testis. There was no significant change in the level of biochemical contents of the testis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 10 days the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.42, 4.60, 146.8, 6.41, 9.45 and 14.3 μg respectively in the testis. There was a significant decrease in the level of the biochemical contents whereas cholesterol level was increased significantly, except there was no significant change in the level of DNA and RNA when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 20 days the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.29, 4.13, 141.2, 6.19, 9.65 and 13.3 μg respectively in the testis. There was a significant decrease in the level of biochemical contents, except cholesterol content where it was increased significantly in the testis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 30 days the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.11, 3.87, 135.5, 5.53, 9.81 and 12.5 μg respectively in the testis. There was a significant decrease in the level of biochemical contents, except cholesterol content where it was increased significantly in the testis when compared with those of the corresponding parameters of the control mice.

The findings of the present study on biochemical contents of the testis revealed that in the mice treated with 4 mg methomyl for 10, 20 and 30 days caused significant decrease in the level of DNA, RNA, protein, glycogen and sialic acid except cholesterol level was
increased significantly. Whereas level of DNA and RNA contents were not changed significantly in the mice exposed to methomyl for 10 days. However, treatment with 4 mg methomyl for 5 days caused no significant change in the levels of biochemical contents of the testis when compared with those of the corresponding parameters of the control mice (Table 1.11; Graph 1.13).

**Testis dehydrogenase and phosphatase enzymes activity** (Table 1.12; Graph 1.14)

In the control mice, the activity of LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 9.06, 12.20, 0.44, 0.94, 7.45, 9.00, 6.10, 18.80 and 14.10 μmoles respectively in the testis. In the mice treated with 4 mg methomyl for 5 days the activity of LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 9.08, 12.12, 0.42, 0.93, 7.43, 8.94, 6.07, 18.70 and 14.12 μmoles respectively in the testis. There was no significant change in the activity of enzymes in the testis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 10 days the activity of LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 9.10, 12.10, 0.37, 0.83, 7.32, 8.90, 6.04, 18.65 and 14.14 μmoles respectively in the testis. There was no significant change in the activity of the enzymes, except 3βHSD, 17βHSD, Na⁺-K⁺ATPase activity where it was decreased significantly when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 20 days the activity of LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 10.14, 11.40, 0.35, 0.75, 7.10, 7.75, 5.80, 17.70 and 14.90 μmoles respectively in the testis. There was a significant decrease in the activity of SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase and ACP activity. However, LDH and AKP activity were increased significantly when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 30 days, the activity of LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 10.30, 11.15, 0.32, 0.72, 6.42, 7.10, 5.42, 16.20 and 15.50 μmoles respectively in the testis. There was a significant decrease in the activity of SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase,
Mg\(^{2+}\)ATPase and Ca\(^{2+}\)ATPase enzymes, whereas LDH and AKP activity were increased significantly when compared with those of the corresponding parameters of the control mice.

Results of the present study on the enzyme activity of dehydrogenase, phosphatase and steroidogenic enzymes in the testis revealed that, in the mice treated with 4 mg methomyl for 20 and 30 days showed significant decrease in the SDH, 3βHSD, 17βHSD, Na\(^+\)-K\(^+\)ATPase, Mg\(^{2+}\)ATPase, Ca\(^{2+}\)ATPase and ACP whereas the activity of LDH and AKP were increased significantly. In the mice treated with 4 mg methomyl for 5 and 10 days caused no significant change in the activity of the dehydrogenase, phosphatase and steroidogenic enzymes, except 3βHSD, 17βHSD, Na\(^+\)-K\(^+\)ATPase activity were decreased significantly in the mice exposed to methomyl for 10 days when compared with those of the corresponding parameters of the control mice (Table 1.12; Graph 1.14).

**Antioxidants and oxidative stress byproducts of the testis in mice on exposure to methomyl (Table 1.13; Graph 1.15)**

In the control mice the level of GSH, ascorbic acid, TBARS and protein carbonyl and was 9.75, 362, 0.24 and 1.24 μg respectively in the testis. In the mice treated with 4 mg methomyl for 5 days, the level of GSH, ascorbic acid, TBARS and protein carbonyl was 9.73, 361, 0.26 and 1.27 μg respectively. There was no significant change in the level of antioxidants and oxidative stress byproducts contents of the testis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 10 days the level of GSH, ascorbic acid, TBARS and protein carbonyl was 9.60, 358, 0.32 and 1.38 μg respectively in the testis. There was a no significant decrease in the levels of antioxidants and oxidative stress byproducts contents, except the level of GSH was decreased significantly when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 20 days the level of GSH, ascorbic acid, TBARS and protein carbonyl was 9.56, 332, 0.37 and 1.43 μg respectively in the testis. There was a significant decrease in the level of GSH and ascorbic acid contents, whereas TBARS and Protein carbonyl were not increased significantly when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 30 days the level of GSH, ascorbic acid, TBARS and protein carbonyl was 8.72, 308, 0.44 and 1.52 μg respectively in the testis.
There was a significant decrease in the level of GSH and ascorbic acid contents, whereas TBARS and protein carbonyl were increased significantly when compared with those of the corresponding parameters of the control mice.

The findings of the present study on biochemical contents of the testis revealed that, in the mice treated with 4 mg methomyl for 20 and 30 days caused significant decrease in the level of GSH and ascorbic acid contents, whereas TBARS and protein carbonyl content was increased significantly. In the mice treated with 4 mg methomyl for 5 and 10 days showed no significant change in the levels of the antioxidants and oxidative stress byproducts of testis, except the level of GSH was significantly decreased in 4 mg methomyl for 10 days treated mice, when compared with those of the corresponding parameters of the control mice (Table 1.13; Graph 1.15).

Oxidative stress enzymes SOD, CAT and GST of the testis in mice on exposure to methomyl (Table 1.13; Graph 1.15)

In the control mice, the activity of SOD, CAT and GST was 42.8, 151 and 4.2 μmoles respectively in the testis. In the mice treated with 4 mg methomyl for 5 days the activity of SOD, CAT and GST was 42.4, 149 and 4.1 μmoles respectively. There was no significant change in the activity of enzymes in the testis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 10 days, the activity of SOD, CAT and GST was 38.6, 146 and 3.5 μmoles respectively in the testis. There was no significant change in the activity of CAT, SOD. However, activity of GST was decreased significantly when compared with that of the control mice.

In the mice treated with 4 mg methomyl for 20 days, the activity of SOD, CAT and GST was 32.8, 133 and 3.1 μmoles respectively in the testis. There was a significant decrease in the activity of CAT, SOD and GST, when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 30 days, the activity of SOD, CAT and GST was 30.1, 122 and 2.6 μmoles respectively in the testis. There was a significant decrease in the activity of SOD, CAT and GST when compared with those of the corresponding parameters of the control mice.
Study on the activity of oxidative stress enzymes in the testis revealed that, in the mice treated with 4 mg methomyl for 20 and 30 days showed significant decrease in the activity of SOD, CAT and GST. However, in the mice treated with 4 mg methomyl for 5 and 10 days caused no significant change in the activity of the enzymes, except the activity of GST was decreased significantly in 4 mg methomyl for 10 days treated mice when compared with those of the corresponding parameters of the control mice (Table 1.13; Graph 1.15).

Biochemical contents of the epididymis (Table 1.14; Graph 1.16)

In the control mice the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.40, 4.52, 220.20, 7.40, 8.40 and 12.20 μg respectively in the epididymis. In the mice treated with 4 mg methomyl for 5 days the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.38, 4.48, 215.6, 7.34, 8.44 and 12.15 μg respectively. There was no significant change in the level of biochemical contents of the epididymis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 10 day the levels of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.36, 4.42, 211.2, 7.28, 8.50 and 12.12 μg respectively in the epididymis. There was no significant change in the level of the biochemical contents, except cholesterol where it was increased significantly when compared with those of the corresponding parameters of the control mice.

In the mice treated with methomyl for 20 days the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.30, 4.08, 199.2, 6.68, 9.60 and 11.40 μg respectively in the epididymis. There was a significant decrease in the level of the biochemical contents, except cholesterol where it was increased significantly when compared with those of the corresponding parameters of the control mice.

In the mice treated with methomyl for 30 days the level of DNA, RNA, protein, glycogen, cholesterol and sialic acid was 2.10, 4.00, 190.1, 6.60, 9.90 and 11.12 μg respectively in the epididymis. There was a significant decrease in the level of the biochemical content, except cholesterol where it was increased significantly when compared with those of the corresponding parameters of the control mice.

Findings of the present study on biochemical contents of the epididymis revealed that, the mice treated with 4 mg methomyl for 20 and 30 days caused significant decrease in
the level of the DNA, RNA, protein, glycogen and sialic acid whereas, cholesterol content 
was increased significantly. However, the mice treated with 4 mg methomyl for 5 and 10 
days, showed no significant change in the level of biochemical contents when compared with 
those of the corresponding parameters of the control mice (Table 1.14; Graph 1.16).

Epididymis dehydrogenase and phosphatase enzymes activity 
(Table 1.15; Graph 1.17)

In the control mice the activity of LDH, SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, 
Ca⁺⁺ATPase, ACP and AKP was 15.20, 14.12, 8.62, 10.70, 6.70, 15.40 and 13.60 μmoles 
respectively in the epididymis. In the mice treated with 4 mg methomyl for 5 days the 
activity of LDH, SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 
15.14, 14.09, 8.58, 10.65, 6.62, 15.34 and 13.62 μmoles respectively in the epididymis. 
There was no significant change in the activity of the enzymes when compared with those of 
the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 10 days, the activity of LDH, SDH, Na⁺- 
K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 15.10, 13.95, 8.50, 10.60, 6.60, 
15.29 and 13.88 μmoles respectively in the epididymis. There was no significant change in 
the activity of the enzymes in the epididymis when compared with those of the 
corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 20 days, the activity of LDH, SDH, Na⁺- 
K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 16.00, 13.20, 7.50, 9.96, 6.16, 
14.60 and 14.70 μmoles respectively in the epididymis. There was a significant decrease in 
SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase Ca⁺⁺ATPase and ACP activity in the epididymis. However, the activity of LDH and AKP were increased significantly when compared with 
those of the corresponding parameters of the control mice.

In the mice treated with 4 mg methomyl for 30 days, the activity of LDH, SDH, Na⁺- 
K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 16.70, 12.84, 7.10, 9.51, 5.96, 
14.32 and 15.36 μmoles respectively in the epididymis. There was a significant decrease in 
SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase and ACP activity. However, the activity 
of LDH and AKP were increased significantly in the epididymis when compared with those 
of the corresponding parameters of the control mice.
Results of the present study on enzymes activity of the epididymis revealed that, in the mice treated with 4 mg methomyl for 20 and 30 days the activity of SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP was decreased significantly, whereas the activity of LDH and AKP were increased significantly. In the mice treated with 4 mg methomyl for 5 and 10 days, there was no significant change in the activity of enzymes of the epididymis when compared with those of the corresponding parameters of the control mice (Table 1.15; Graph 1.17).

**Serum testosterone level (Table 1.16; Graph 1.18)**

The level of serum testosterone was 52.40ng/ml in the control mice. The mean serum testosterone level with an effective dose 4 mg methomyl treatment for 5, 10, 20 and 30 days was 51.38, 40.70, 31.80, 20.62 ng/ml respectively. There was a significant decrease in the level of the serum testosterone in mice treated with methomyl for 10, 20 and 30 days. However, 5 days methomyl exposure caused no significant change in the level of serum testosterone when compared with that of the control mice (Table 1.16; Graph 1.18).
DISCUSSION

a) Effect of methomyl on testes and accessory reproductive organs in albino mice

Humans are exposed to a vast array of chemicals through a wide range of portals. Innumerable environmental, toxic hazards arising from nature have always existed but the additional exposures to man-made chemicals have increased progressively since the industrial revolution. Regulatory agencies have developed licensing guidelines for new chemicals in order to protect human populations from damaging environmental exposures, which are an integral part of our built environment (Martin et al., 2010). Indeed, a recent, comprehensive review has listed published data on over 600 chemicals to which humans can be exposed that affect the male reproductive system (Krause, 2008). The testis of human and other mammals are highly susceptible to damages caused by genetic disorders, environmental or occupational exposure to chemicals or by other means. Specific causes of testicular damage have been catalogued by several workers (Jackson and Ericsson, 1970; Lucier et al., 1977), although these listings are by no means complete.

Spermatogenesis is a complex process and an extremely ordered process in which spermatogonial stem cells form spermatozoa. In the light of recent technological advancements that have helped us to better understand spermatogenesis. These include the use of proteomics and genomics (Calvel et al., 2010) and mouse genetic models (Verhoeven et al., 2010) to examine different aspects of spermatogenesis. There are also other facets of spermatogenesis that have gained momentum in recent years such as regulation of apoptosis (Shaha et al., 2010); roles of oestrogens (Carreau et al., 2010; Carreau and Hess 2010), aromatase (Carreau et al., 2010) and androgens (Verhoeven et al., 2010; Walker, 2010) in spermatogenesis; non-genomic action of testosterone and its impact on spermatogenesis (Walker, 2010); regulation of mitosis and meiosis during spermatogenesis (Wolgemuth and Roberts 2010); impact of environmental toxicants and lifestyle effects on spermatogenesis (Sharpe, 2010); transcriptional regulation of spermatogenesis (Bettegowda and Wilkinson, 2010).

Spermatogenesis and steroidogenesis are the major functions of testis. Testis is a complex organ containing three important cell types such as germ cells, Sertoli cells and Leydig cells in close proximity with unique autonomic and vascular features regulated by endocrine, paracrine and autocrine mechanisms. Spermatogenesis occurs in mitotic, meiotic
and postmeiotic phases. In the mitotic (proliferative) phase, spermatogonia undergo either self-renewal or differentiation, both involving successive divisions (Eddy, 2002). Mitotic germ cells (spermatogonia) are located in the basal compartment, whereas meiotic and postmeiotic germ cells are found in the luminal compartment. In the meiotic phase, genetic materials are recombined and segregated in spermatocytes. In the postmeiotic phase, spermatids undergo a series of morphological and structural changes to become spermatozoa. These changes include acrosome and tail formation, chromosome condensation and the removal of the excessive cytoplasm at the time of spermiation. The entire process of spermatogenesis takes about 35 days in the mouse, with mitotic, meiotic, and postmeiotic phases lasting 11, 10 and 14 days, respectively (Eddy, 2002; Baoan et al., 2005). The anterior pituitary participates in the control of both these functions through secretion of gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Steinberger and Steinberger, 1975; Sharpe, 1987). Luteinizing hormone (LH) secreted by the anterior pituitary gland under stimulation from the hypothalamus, acts on the Leydig cells within the testis to induce the synthesis and secretion of testosterone which allows normal spermatogenic development. In testes, testosterone is synthesized almost exclusively within Leydig cells (Ewing and Zirkin, 1983; Neaves, 1977). A block in the production and release of testosterone can occur either at the site of the Leydig cells or via an effect on the pituitary or hypothalamus by inhibiting the release of LH (Martin et al., 1998).

Hormonal imbalances also are important factors in the development of focal proliferative lesions of Leydig cells, including increased estrogenic steroids in mice and hamsters and elevated pituitary gonadotrophins resulting from the chronic administration of androgen receptor antagonists, 5α-reductase inhibitors, testosterone biosynthesis inhibitors, GnRH agonists, and aromatase inhibitors (Clegg et al., 1997; Cook et al., 1992, 1999). Many xenobiotic chemicals when administered chronically to rats disrupt the hypothalamic–pituitary–testis axis at one of several possible sites interfering with negative feedback control, resulting in an over production of luteinizing hormone (LH) that causes the proliferative changes (hyperplasia, adenoma) in Leydig cells. The hypothalamic-pituitary-gonadal axis is important toxicological perspective, this arrangement creates even more sites where toxic responses may have an impact on reproduction (Silverman et al., 1994; Hotchkiss and Knobil, 1996; Robert et al., 2000).
Biology of Leydig cells is an essential prerequisite to understanding male fertility, sexual function, and reproductive health. The primary function of the Leydig cells is the biosynthesis and secretion of testosterone, the principal circulating androgen, the synthesis and secretion of testosterone from the Leydig cells is under the control of the pituitary luteinizing hormone (LH), which in turn is regulated by luteinizing hormone releasing hormone (LHRH). The biosynthesis of testosterone involves numerous reactions and requires several enzymes and cofactors that carry out the process in the mitochondria, as well as in the microsomes. Testosterone or its metabolites play an important role in the support of sexual behaviour and in maintaining the reproductive tracts including the maintenance of spermatogenesis (Sundaram and Witorsch, 1995). Estrogens also have anti-androgenic properties in the males causing azoospermia and reduction of plasma testosterone levels (Verjans et al., 1974; Hunt et al., 1979), another mechanism of action of estrogenic substances is inhibiting the testicular steroidogenesis (Samuel et al., 1967; Oshima et al., 1967). In certain cases higher doses of estrogens are known to inhibit the male reproductive function. (Samuel et al., 1964, 1967; Kalra and Prasad, 1969). The target organs for estrogens of any origin in the male are the testis, epididymis, vas deferens, seminal vesicles and prostate gland (Stupf et al., 1971; Van Beurdan-Lamers et al., 1974).

Epididymides are long, coiled tubes that rest on the backside of each testicle. It functions in the transport and storage of the sperm cells. Epididymides are important because they provide suitable environment for morphological and biochemical changes in spermatozoa (Orgebin-Crist, 1969). The spermatozoa attain their maturity and fertility capacity during their passage in the efferent ducts and highly convoluted single tabular epididymis. It has been stated that the long epididymis provides suitable environment for the spermatozoa to undergo fertilization. The epididymis provides a favourable milieu for the acquisition of fertilizing ability, motility, storage and survival of the spermatozoa (Jehan et al., 1973; Brooks, 1981). Physiological and biochemical integrity of epididymal canal is dependent on androgens (Brooks, 1974; Setty et al., 1977). It has been reported that epididymis performs both secretory and absorptive functions, sperm maturation takes place because of the proteins synthesized and secreted by epididymal tissue (Klinefelter and Hamilton, 1985). Brooks (1981) has reported that androgen deficiency causes a marked reduction in the tubular diameter, a general regression of epididymal epithelium, a rapid decline in the number of spermatozoa within the cauda epididymis and changes in the composition of the epididymal plasma. Similar effects have been reported in rats treated with lindane (Sujatha et al., 2001).
In the present investigation, on testes and accessory reproductive organs weight revealed that treatment with 3 and 4 mg methomyl caused significant decrease in the weight of testes and accessory reproductive organs such as epididymides, vasa deferentia, seminal vesicles, prostate gland, coagulatory glands and Cowper's glands. However, treatment with 1 and 2 mg methomyl caused no significant change in the weight of the testes and accessory reproductive organs, except seminal vesicles, prostate gland and coagulatory glands weight where they were decreased significantly with 2 mg methomyl treatment in mice. Durational exposure study with 4 mg methomyl for 10, 20 and 30 days caused significant decrease in the weight of testes, epididymides, vasa deferentia, seminal vesicles, prostate gland, coagulatory glands and Cowper's glands, except seminal vesicles weight was not changed significantly in the mice exposed to methomyl for 10 days. However, methomyl exposure for 5 days caused no significant change in the weights of the testes and accessory organs.

A number of environmental chemicals have been implicated in the significant decline in the mean sperm count from $113 \times 10^6$/mL in 1940 to $66 \times 10^6$/mL in 1990 among men without a history of infertility (Carlsen et al., 1992; Kniewald et al., 2000). Quality of sperm production has been adversely affected due to the exposure of certain drugs and chemicals, particularly mutagens and teratogens, pesticides can be translocated, bioconcentrated or converted into more dangerous chemicals (Matsumara et al., 1972). There are some reports of varying degree of testicular dysfunction in pesticide factory workers such as oligospermia, azoospermia, degeneration of germinal epithelium in testicular biopsies and elevated serum levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Potashnik et al., 1978; Whorton et al., 1979). There is a clear correlation between the degree and duration of exposure to pesticides, the extent of spermatogenic arrest and hormonal imbalance.

There are several possible mechanisms for anti-gonadal actions of toxicants. It has been reported that, organophosphate insecticide quinolphos has direct effect on acetyl cholinesterase (AChE), resulting in alteration in the pituitary gonadotropins and could influence testicular function directly through its effect on the pituitary AChE or they may change the concentration of the neurotransmitters (Sarkar et al., 2000). Pesticides induce inhibition of the acetyl cholinesterase (AchE) which in turn might increase the accumulation of acetyl choline (Ach) in the pituitary and hypothalamus, which in the complex circuitry of neuroendocrine regulation can invariably affect a secondary
transmitter, especially dopamine or 5 hydroxytryptamine (5-HT) (Corrodi et al., 1967; Butcher, 1969; Robinson, 1983; Bradford, 1986). Ferguson et al., (1984) have reported that the treatment with a carbamate pesticide carbofuran inhibits acetylcholinesterase (Ache), resulting in alterations in the pituitary gonadotropins and could influence on gonadal function directly through the effect on the pituitary Ache in rats. This may be due to imbalance in gondadal steroids which are essential for normal functioning of the gonads (Sharpe, 1987).

Yousef et al., (1995) have found affected sperm quality and reduced sperm count in the rabbits treated with a carbamate pesticide carbofuran. Carbofuran treatment orally to adult rats with the doses ranging 0.2 to 0.8 mg/ kg five times per week for 60 days caused dose dependent decrease in body weight, reduction in the weight of epididymis, seminal vesicles, ventral prostate and coagulatory gland (Pant et al., 1995). Total sperm count and sperm motility has been decreased with increased number of abnormal sperms in the rats exposed to carbofuran exposure. Histopathological examination revealed atrophied seminiferous tubules and degenerative changes of sertoli cells at 0.4 mg/ kg/ day (Pant et al., 1997). Recently, Gallegos et al., (2010) have reported that, chronically exposed men by carbofuran revealed reduced sperm concentration, binucleated spermatozoa (26%) and of multinucleated spermatids (10%). Carter et al., (1984) have found oral administration of benomyl in the pubertal and post pubertal rats resulted in decreased testicular weight and decreased sperms in the epididymis and vas deferens. Recently, Yu et al., (2009) reported that, rat receiving 100 and 200 mg/kg (bw), oral gavage of carbendazim for 80 days showed atrophic testes and epididymides, marked histopathological abnormality of the testis, reduced weight of the testis and epididymis, and decreased sperm count and motility. The carbamate fungicides, Maneb and Zineb are known to cause testicular atrophy with damaged germinal epithelium and reduced sperm motility and viability (Lucier et al., 1977; Rao and Schwetz, 1982).

In the present study with increasing dose and prolong exposure of methomyl caused decrease in testes, epididymides, vasa deferentia, seminal vesicles, prostate glands, coagulatory glands and cowper’s glands weight may be due to a) antigonadal action of pesticide or deprived levels of androgens, which are very much essential for normal functioning of accessory reproductive organs (Mann, 1974; Anderson et al., 1982; Jana et al., 2003) or by affecting either the hypothalamus or pituitary or both (Stoker et al., 2000; Okazaki et al., 2001; Lathoumycandang et al., 2002). b) Germ cell loss and testicular
atrophy, a valuable index of reproductive toxicity in male animals (Amman, 1992), as seen in other experimental conditions (Chapin and Lamb, 1984; Hess et al., 1988; Nakas et al., 1992; Narayana et al., 2000). Decrease in the activity of acetylcholinesterase as methomyl is a well known inhibitor of acetylcholinesterase (Lohitnavy and Sinhaseni 2006; Makrides et al. 2005; Garg et al., 2009), and c) some authors (Mohgoub and El-medony, 2001) have reported that chronic exposure of methomyl (17 mg/kg for 2 months) to male rat reproductive system caused significant decrease in level of testosterone, and other hormonal imbalance and histopathology resulted in degeneration of tissue structure and seminiferous tubules upto cellular destruction, this may have some relation to inhibited acetylcholinesterase in the testis or brain (Maitra and Sarkar, 1996). Similarly, decreased acetylcholinesterase activity was observed along with severe testicular damage in quinolophos treated rat also (Sarkar et al., 2000). Decrease in the level of testosterone, lowered sperm density, degenerative changes and decreased protein quantity (Mathew et al., 1992; Nin et al., 2003; Jana et al., 2003; Pant et al., 2003; Poon et al., 2004; Narayana et al., 2005).

b) Effect of methomyl on the number and diameter of spermatogenic cells (Spermatogonia, spermatocytes and spermatids) and Leydig cells in albino mice

Many of the reproductive toxicants have primary effects on the testis, which potentially overshadow effects downstream on the efferent ductus and epididymis. Thus, studies in which testicular atrophy occurs after chronic or subchronic exposures can lead to permanent infertility. Recent studies have confirmed that chemicals can also alter the time required for sperm transport through the epididymis. A search of the literature for the period 1995-1997 showed that testis has been the major emphasis in toxicology. Reports on male reproductive effects by earlier investigators reveals the testicular toxicity reflected by different ways such as biochemical effects, histopathology, impaired neurotransmitter metabolism and hormonal imbalance (Lucier et al., 1977; Kitagawa et al., 1977; Raizada et al., 1979; Carter et al., 1987; Somkuli et al., 1987; Ray et al., 1987, 1991; Song et al., 1988; Toppari et al., 1990; Wisslings et al., 1990; Chapin et al., 1990; Goldman et al., 1990; Dickerson and Safe, 1992; Sharpe and Skakkeback, 1993; Goldman et al., 1994; Maitra and Sarkar, 1995; Whitney et al., 1995; Chanda and Pope, 1996; Mathsson et al., 1996; Akbarsha and Sivaswamy, 1997; Dam et al., 1998; Shrivastava and Shrivastava, 1998; Dam et al., 1999; Hunter et al., 1999; Sarkar et al., 2000; Andrews et al., 2001; Usha et al., 2003; Ryan T. Good et al., 2004; Sharpe and Irvine, 2004; Yu et al., 2004; Suresh et
al., 2005; Narayana et al., 2005a; Narayana et al., 2006; Prashanti et al., 2006; Kuldip Jana et al., 2006; Pina-Guzman et al., 2006; Amina et al., 2007). It has been found that pesticides cause formation of defective seminiferous tubules. The defective seminiferous tubules possess giant cells with significantly less spermatogenic cells and lumen with loss of sperm reflecting hypospermatogenic condition or spermatogenic inhibition, immature sloughed off germ cells in the lumen, disturbed testosterone function. Similar types of effect have been reported in the rats treated with carbamate pesticides Zineb and Mancozeb (Raizada et al., 1979; Kackar et al., 1997; Ksheerasagar and Kaliwal, 2003, 2010). Formation of giant cells also has been reported in rats treated with organophosphate insecticide phosphomidon (Akbarsha and Sivaswamy, 1997, Shreelaxmi, 2007).

In the present investigation, study on number and diameter of spermatogenic cells and Leydig cells revealed that there was a significant decrease in the number and diameter of spermatogenic and Leydig cells with 2, 3 and 4 mg methomyl treatment except with 2 mg there was no significant change in the number of primary spermatocytes, Leydig cells and diameter of spermatogonia. However, treatment with 1 mg methomyl showed no significant change in the number and diameter of spermatogenic and Leydig cells. The durational exposure study with 4 mg methomyl for 10, 20 and 30 days caused significant decrease in the number and diameter of spermatogenic cells and Leydig cells, except the number of Leydig cells and diameter of primary spermatocytes were not changed significantly in the mice treated with 4 mg methomyl for 10 days, whereas treatment with 4 mg methomyl for 5 days caused no significant change in the number and diameter of spermatogenic and Leydig cells. Similar results have been obtained in mice with treatment of mancozeb, carbosulfan, phosphomidon and Indoxacarb (Ksheerasagar and Kaliwal, 2005, 2010; Ksheersagar, 2005; Shreelaxmi, 2007; Mudaraddi, 2009). Poon et al., (2004) have reported that pentylether, 1, 4-diethoxybutane and 1, 6-dimethoxyhexane caused decreased sperm density in the epididymis which is an indicator of reduced spermatogenesis. It has been reported that Archlor is a complex mixture of polychlorinated biphenyl (PCB) inhibits steroidogenesis in male rats (Andric et al., 2000). Shivanandappa and Krishnakumari (1983) have reported in the rats treated with hexachlorocyclohexane (HCH) caused testicular atrophy and showed shrunken seminiferous tubules, filled with mass of degenerating spermatocytes and spermatids, which appears as bi-and multi nucleated, Leydig cells showed significant degeneration with pycnotic nuclei and reduced nuclear size. It has been suggested that the effect on male germ cell population brought about by mainly germ cell apoptosis causing extensive decrease in the number of germ cells

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and secondly by chromosomal damage due to inhibition of DNA synthesis leading to formation of giant cells. In the present study decrease in the number and diameter of spermatogenic cells may be due to reduced spermatogenesis.

Further, it has been reported that methyl parathion is known to induce sperm shape abnormalities and decreased sperm count in rodents with positive correlation with decreased ascorbic level in the testis (Mathew et al., 1992; Narayana et al., 2005). Methyl parathion also caused major defects as epithelial sloughing, multinucleated giant cells, necrosis of seminiferous epithelium, cellular degeneration, nuclear pyknosis, and finally the tubular atrophy which resulted in very highly significant reduction in seminiferous tubular diameter (STD) and seminiferous epithelial height, and significant decrease in volumes of different types of germ cells in rats (Narayana et al., 2006). Amina et al., (2007) have reported in male mice treated with dimethoate caused sperm production and percent motile sperms. Narayana et al., (2006) have reported methyl parathion an organophosphate pesticide caused structure and function of accessory reproductive organs in the rat. Pina-Guzman et al., (2006) have reported that methyl parathion exposure altered nuclear chromatin structure and DNA damage in spermatozoa are implicated as possible causes of increased infertility in males and has serious impact on embryo development. Prashanti et al., (2006) have reported that methyl parathion treated male rats showed deterioration in the structural integrity of the reproductive organs and also the biochemical parameter in the epididymis. Several studies in men and animals have demonstrated that organophosphorous (OP) compounds alter male reproductive function, particularly semen quality and hormone balance (Padungtod et al., 1999; Padungtod et al., 2000; Sanchez-Pena et al., 2004; Narayana et al., 2005; Pina-Guzman et al., 2005). Recio et al., (2001) observed similar results in agricultural workers exposed to a mixture of OP, including methyl parathion. Meeker et al., (2004a) suggests that environment exposure to chlorpyrifos is associated with increased DNA damage in human sperm, and results from disrupted chromatin structure. Sanchez-Pena et al., (2004) and Pina-Guzman et al., (2005) have shown that sperm chromatin structure is a target of OP exposure in humans and animals. Similarly Alwachi et al., (1999) have observed fall in the number of spermatogonia and primary spermatocytes and spermatid conversion process with high dose of nicotine treatment. In the present study decrease in the number and diameter of spermatogenic cells may be due to methomyl might have affected testicular chromatin structure.
Further, carbamate insecticide carbaryl is known to cause sperm abnormalities, reduction in number of spermatogonia, spermatozoa and degeneration of Leydig cells in rats and mice (Degrave et al., 1976; Kitgawa et al., 1977; Shrivastava and Shrivastava, 1998). Pant et al. (1995) have reported carbofuran a carbamate pesticide causes dose dependent reduction in number of epididymal sperm count and sperm motility with increasing abnormal sperms number. Good et al., (2004) have reported that carbofuran caused significant changes in endocrine hormones progesterone, cortisol, and testosterone. It has been reported that, administration of Carbamate fungicide, Carbendazim through oral route of single dose (400 mg/kg) caused selective loss of spermatids, asynchrony of the stages in the spermatogenic cycle and development of Sertoli cell fibrosis of the seminiferous tubules in rats (Kadalmani et al., 2002). Carbendazim also induces chromosome aberrations in spermatids with a high incidence of aneuploidy (Matsuo et al., 1999). Mohgoub and El-medony (2001) have reported that chronic exposure of methomyl (17 mg/kg for 2 months) to male rat caused significant decrease in the level of testosterone and increased in the levels of FSH, LH and prolactin with histopathological alteration like degeneration of tissue structure and seminiferous tubules upto cellular destruction, and suggested that, chronic exposure to methomyl insecticide has deleterious effects on rat testes. In present study with increase in dose and duration there is decrease in the level of testosterone. Thus, in the present study decrease in the number and diameter of spermatogenic cells may be due to reduced spermatogenesis and methomyl might have caused deleterious effects on testes which may resulting in disruption of spermatogenic cells by apoptosis or necrosis.

Apoptosis is a physiological process of cell death leading to the controlled elimination of single unwanted cell from the midst of a viable tissue without damaging the neighbouring unaffected cells (Pesce et al., 1993; Averal et al., 1996). Growth factors and cytokines are also involved in local control mechanism influencing testicular apoptosis through paracrine and autocrine mechanisms. Intra testicular androgens, secreted by Leydig cells, also play an important paracrine role in preventing germ cell degeneration (Billig et al., 1996; Topanainen et al., 1993). The elevation of testicular temperature or other change associated with cryptorchidism may cause germ cell apoptosis (Yin et al., 1998). Severe spermatogenic inhibition and disruption of microtubule function in Sertoli cells leading to testicular damage has been reported by Carter et al., (1987), Boekelheide et al., (2003), Nakai et al., (1992), Markelewicz et al., (2004) in the rats treated with a carbamate fungicide carbendazin. Histologic examination of testis 245 days post exposure revealed
severe seminiferous tubular atrophy (>85%). These seminiferous tubules showed Sertoli cells only with thickened basement membrane. Once a tubular basement membrane has thickened, that portion of the tubule may no longer be available for normal spermatogenesis. It has been observed that members of carbamate pesticides such as disulfiran and its metabolite dithiocarbamate, can interfere with catecholamine neurotransmitter metabolism by inhibiting the activity of dopamine β-hydroxylase (DβH) this is an enzyme that converts dopamine to norepinephrine and the norepinephrine then stimulates the release of GnRH. Thus GnRH release is affected through the inhibition of DβH (Maj and Vetulani, 1969; Prezewlocka et al., 1975). The mechanism plays an important regulatory and/ or modulatory role in brain hypothalamic control of pituitary luteinizing hormone (LH) release (Kalra and Kalra, 1983). In rats administration of N-methyl dithiocarbamate causes suppression of LH surge by interfering with catecholamine activity (Goldman et al., 1994). Goldman et al., (1990), have reported that the insecticide, chlordimeform may destroy the endocrinologic homeostasis by suppressing the release of GnRH. It has been revealed that the toxic agents may act directly on the gonadotropins to alter the synthesis of gonadotropins and their secretion or indirectly GnRH or to the gonadal steroids. Both the actions will result in the alterations in the serum levels of FSH and LH (Dickerson and Safe, 1992).

In the present study, histologic observations of the testis of the mouse treated with 1 mg methomyl revealed decrease in the number of spermatocytes, spermatids and sperms in the lumen of seminiferous tubules and interstitial tissue contains clusters of Leydig cells. The testis of the mouse treated with 2 mg methomyl exhibited decrease in the number of spermatogenic cells, formation of giant cells, vacuoles and loss of number of sperms in the lumen of the seminiferous tubules with disturbed architecture of Leydig cells. The testis of the mouse treated with 3 and 4mg methomyl showed formation of giant cells, loss of spermatogenic cells and lumen with decreased number of sperms. Leydig cells are in deformed condition. Seminiferous tubules are atrophied. Lumen showed tissue debris and clumped sperms.

Histologic observations of the testis of the mouse treated with 4mg methomyl for 5 days showed abnormal spermatogonial arrangement. The seminiferous tubules are closely packed, there was loss of number of spermatocytes, spermtids and sperms in lumen. The tubular spaces are packed with interstitial tissue. The testis of the mouse treated with 4mg methomyl for 10 days showed seminiferous tubules with disturbed spermatogenesis.
Lumen of the seminiferous tubules exhibits loss of sperms. Leydig cells are in deformed condition. The testis of the mouse treated with 4mg methomyl for 20 and 30 days showed formation of giant cells, vacuoles, and decreased number of spermatogenic cells and lumen with loss of sperms. Seminiferous tubules are atrophied. Leydig cells are in deformed condition.

In conclusion, present investigation indicates, with high dose and prolong exposure of methomyl affect the spermatogenesis showing antispermaticogenic and anti-androgenic property as reflected by the effect on number and diameter of spermatogenic and Leydig cells, testes and accessory reproductive organs weights as testicular steroids influences spermatogenesis and function of accessory reproductive organs (Setty et al., 1977; Sharpe, 1987; Brooks, 1987; Kumar et al., 1992; Stroker et al., 2000; Okazaki et al., 2001; Luchoumycandane et al., 2002; Jana et al., 2003; Poon et al., 2004; Narayana et al., 2005; Narayana et al., 2006; Prashanti et al., 2006). The other possibility might be due to germ cells apoptosis and chromosomal damage resulting into decreased number of germ cells and formation of giant cells (Shivanandappa and Krishnakumari, 1983; Chapin et al., 1983; Carter et al., 1987; Bhatnagar and Soni, 1990; Akbarsha and Sivaswamy, 1997; Hess et al., 1998; Usha et al., 2003; Pina-Guzman et al., 2006; Prashanti et al., 2006). The effect may be due to deprived level of androgens mediated through the gonadotropins of the pituitary due to the effect on hypothalamus (Goldman, 1990; 1994; 2009; Maitra and Sarkar et al., 1996; Sarkar et al., 2000). Radad et al., (2009) reported that, Sprague-Dawley rats treated orally with methomyl (2 mg/kg b.w.), three times weekly for three months caused necrosis of seminiferous tubular cells and formation of intratubular giant cells in testes. It has also caused other histopathological alteration in liver, kidneys, lungs, and spleen and they concluded that methomyl was found out to be potentially toxic to liver, kidneys, lungs, testicles and spleen. Similar reports have been reported with phosphomidon and mancozeb in albino mice (Shreelaxmi. 2007; Ksheerasagar and Kaliwal, 2003, 2010). Thus it is presumable that the testicular damage induced in the present study might have relation with decreased acetylcholinesterase activity or deprived level of androgens mediated through the gonadotropins of the pituitary due to the effect on hypothalamus (Goldman et al., 2009). This could be expected to affect steroidogenesis and spermatogenesis on methomyl treatment.
c) Effect of methomyl on biochemical contents (DNA, RNA, protein, glycogen, cholesterol and sialic acid) in testis and epididymis of albino mice

Nucleic acids

DNA functions by providing a blueprint for the synthesis of proteins from individual amino acids, and these proteins, in turn, carry out all functions of the cell, whether it be generation of cellular energy (ATP) or building other macromolecules of the cell such as proteins, lipids and complex carbohydrates. Transcription involves the synthesis of strands of RNA strands, thereby it taking part in the process of translation. The specific functions of different proteins are dictated by the three-dimensional shape of the protein, which in turn is determined by the sequence of amino acids that make up the protein. This sequence of amino acids, in turn, is determined by the particular sequence of bases in the DNA molecule directed through RNA. The specific sequence of three nucleotides codes for one particular amino acid. Since there are 43 possible triplet combinations, or codons, of the four nucleotides, the genetic code has 64 possible combinations. However, there are only 21 amino acids, so most amino acids have two or more codons in the genetic code (Bruce et al., 2002; Nelson and Cox, 2008).

DNA damage in an eukaryotic cell will results in an immediate repair of the lesion using a convenient repair mechanism, unexpected cell death (necrotic), formation of a cancerous cell or apoptosis (Vinson and Hales, 2002). Thus mutations that occur in the DNA of germinal cells are of critical importance because they can be passed on to future generations. Thus, all hereditary diseases are a result of an acquired mutation in a sperm or egg cell that occurred in a preceding generation. Of course, if the mutation occurs in a gene that is required for the survival of the germ cell itself, then it cannot become a heritable mutation because the cell will die and thus be unable to pass on the mutated DNA during fertilization. It is also possible that a germinal mutation will result in loss of a vital gene necessary for the survival of the fertilized egg (Baarends et al., 2001; Olsen et al., 2005; Longley et al., 2005; Wood et al., 2005; Jaroudi and SenGupta, 2007). Spermatogonial cells are susceptible to genotoxic agents coming from endogenous metabolites or environmental factors that may lead to various types of DNA damage. A great number of divisions in the spermatogonial stem cells occur at the beginning of spermatogenesis. This high level of mitotic activity may cause an increase in the formation of damaged sites in the...
nuclear genome originating from DNA replication machinery errors (Evenson et al., 2002; Ozturk and Demir, in press).

In the present graded dose exposure study, there was a significant decrease in the levels of DNA and RNA in the testis and epididymis in the mice treated with 3 and 4 mg methomyl. However, treatment with 1 and 2 mg methomyl showed no significant change in the levels of nucleic acids in the testis and epididymis, except RNA where it was decreased significantly in the testis with 2 mg methomyl treatment. The durational exposure study for 30 days treatment with an effective dose 4 mg methomyl revealed a significant decrease in the level of DNA and RNA in the testis and epididymis. However, treatment with 4 mg methomyl for 5 and 10 days caused no significant change in the levels of nucleic acids in the testis and epididymis. Similar results have been made in rats exposed to Lindane, carbosulfan, phosphamidon, Indoxacarb and monocozeb (Sujata et al., 2001; Ksheerasagar, 2005; Shreelaxmi, 2007; Mudaraddi, 2009; Ksheerasagar and Kaliwal, 2003, 2010). Some of the pesticides are known to cause genotoxic effects in exposed organisms. The main toxic effect of pesticides is the inhibition of acetylcholinesterase (AchE), but other mechanisms are responsible for their genotoxicity, such as those related to their alkylating (Dedek et al., 1984; Mehl et al., 2000) and phosphorylation properties (Pina-Guzman et al., 2005).

It has been reported that organophosphorous pesticides such as chlorophyrifos, acephate, phosphamidon, monocrotophos, methyl parathion and carbamate pesticides such as capton, furdan, carbosulfan, aldicarb, benzylmethyl, carbofuran and nitrosopropoxur cause various chromosomal aberrations such as chromosome breaks, dots, deletions and laggards and are able to decrease the RI (Replication Index) by preventing the replication of DNA at S-phase of the interphase nucleus (Adhikari and Grover, 1988; Rupa et al., 1991; Topktas et al., 1996; Saxena et al., 1997; Rahman et al., 2002). Marchetti et al., (2001) found structural aberrations and aneuploidy in spermatocytes from male mice treated with an agent used in cancer thermotherapy, effects that were transmitted to the zygotes. Similarly RNA synthesis is known to be altered by chemical action. An increase in the synthesis of RNA is one of the first metabolic alterations brought about by testosterone or dihydrotestosterone and precedes the increase activities of many enzymes. RNA synthesis would in turn influences the level of protein synthesis. Thus the total RNA content of an organ is an index of functional status (Coffey et al., 1968). It has been reported that administration of Lindane (5 mg/ kg/ day) for 30 days inhibited synthesis of DNA, RNA
and proteins in testis of rats (Sujatha et al., 2001). The carbamate pesticide carbaryl rapidly inhibited DNA, RNA and protein synthesis in L-2 cells from rat lung (Lockard et al., 1982).

Adverse effects, ROS also induce lipoperoxidation that changes membrane permeability; it leads to protein impairment, enzyme inactivation and at the end to DNA damage. Plasmatic membranes of spermatozoa contain high concentrations of polyunsaturated fatty acids and therefore, are highly sensitive to oxidative stress (Rao and Shaha, 2000). The oxidative stress in testicular tissue and measured the level of malondialdehyde (MDA), it has been shown that several pesticides causes reactive oxygen species (ROS) and causes production of malondialdehyde is one of the most important products of lipid peroxidation and interferes with protein biosynthesis by forming adducts with DNA, RNA and protein (Doreswamy, 2004). There are many reports that altered nuclear chromatin structure and DNA damage in spermatozoa and are implicated as possible causes of increased infertility in males (Sanchez-Pena et al., 2004; Pina-Guzman et al., 2005; Pina-Guzman et al., 2006).

In addition, it has been recently reported that carbamate pesticides maneb (Ahmaed et al., 2010), N,N-diethylthiocarbamate (Valentine et al., 2010), methyl thiophanate (saquib, 2010), aldicarb, propoxur (Maran, 2010; Mehta et al, 2010), disulfiram (Morrison et al., 2010), Methiocarb (Ozeden and Alpertunga, 2010), thiram (Rana and shivandappa, 2010), carbaryl (Ferrary, 2010), carbosulfan (Rai et al., 2010) induce oxidative stress. As a result of oxidative attack in spermatozoa different types of DNA lesions have been demonstrated including single and double DNA strand breaks, cross-links, chromosomal aberrations and DNA base oxidation (Emerit, 1994; Hughes et al., 1996; Lopes et al., 1998; Nackerdien et al., 1991; Twigg et al., 1998). Meeker et al., (2004 a, b) found that environmental exposures of OP can be associated with increased sperm DNA fragmentation. Among the potential molecular mechanisms of genotoxicity of OP and carbamate compounds are induction of oxidative stress and alkylation. Oxidative stress has been associated to chromatin cross-linking, DNA strand breaks, DNA base oxidation and chromosomal aberrations (Emerit, 1994). Debnath and Mandal (2000) observed that testicular damage caused by quinolphos was due to free radical mediated lipid peroxidation (LPO). Padungtod et al., (1999) and Racio et al., (2001) have reported that chromosomal damage expressed as increased frequency of an euploidy after occupational exposure to a mixture of OP. Pina-Guzman et al., (2006) have reported that altered nuclear chromatin structure and DNA damage in spermatozoa are implicated as possible causes of increased
infertility in males. It has been reported that organophosphorous pesticides methyl parathion induce mutation in bone marrow cells of rats and mice and sperm shape abnormalities in mice (Grover and Malhi, 1985; Mathew et al., 1990, 1992).

It has been reported that the methomyl formulation Lannate® 25 induces DNA damage in liver and kidney and induces micronucleus in bone marrow cells of Swiss CDI mice with formulation of reactive oxygen species (Bolognesi et al., 1994). Hence, in the present study the reason for decreased nucleic acids level on influence of methomyl treatment in mice may be due to disturbed cell division (Hemavathy and Krishnamurthy, 1987; Topktas et al., 1996) or due to cell damage by increased production of ROS (Kim et al., 1997; Lohitnavy and Sinhaseni, 1998; McCarrol et al., 2002). Therefore, in the present study, the decrease in the levels of nucleic acids in testis and epididymis under the influence of methomyl treatment in mice may be due to (a) Decrease in mitotic index and disturbed cell division (Fessel et al., 1993; Topktos et al., 1996; Akbarsha and Sivaswamy, 1997; Emeritt, 1994), (b) Oxidative stress as a result of which synthesis of macro molecules are reduced, different types of DNA lesions found (Nackerdien et al., 1991; Emeritt, 1994; Hughes et al., 1996; Lopes et al., 1998; Twigg et al., 1998; Banerjee et al., 1999; Ranjbar et al., 2002; Debnath et al., 2002; Sharma et al., 2005; Pina-Guzman et al., 2006) and normal metabolic process was altered and caused oxidative damage to important macromolecules such as DNA, RNA, protein and lipids and lead to physiological attritions (Eun-Sun Hwing and Gun-Hee Kin, 2007). (c) Effect on CNS (Central Nervous System) may suppress the brain’s release of gonadotropic hormones, FSH and LH through their effect on acetyl cholinesterase activity (Nagvi et al., 1992; Lyons, 1999; Sarkar et al., 2001). (d) Inhibition of DβH (Dopamine-β-hydroxylase) and release of GnRH and gonadotropins (Maj and Vetulani, 1969; Prezewlocka et al., 1975) thereby affecting the production of gonadal steroids. The synthesis of RNA is potentially influenced by testosterone or dehydrotosterone (Coffey et al., 1968). Therefore, the decreased DNA and RNA in the testis and epididymis also may be due to genotoxic action or oxidative stress of methomyl or decreased levels of testosterone through its effect on central nervous system and gonads.

Proteins and Glycogen

Proteins are building blocks of amino acids, ultimately product directed by the RNA through central dogma of translation. Proteins function in different ways in the cell as
enzymes, structural proteins receptors, hormones and some antibodies (Bruce et al., 2002). Enzymes catalyse all cellular reactions, which make them extremely important in cell activity. Structural proteins are major constituents of skeletal and muscular tissue and also cell membranous structures contain a structural component (Nelson et al., 1989). Insufficient amount of protein in diet may lead to a total protein loss resulting in malignant disease of stomach, intestine, pancreas and peptic ulcer in enteritis. The decrease in protein content may be due to the intestinal or pancreatic dysfunction resulting in malabsorption, which leads to malnutrition (Kaneko et al., 1996). Significant decrease in protein level might be due to catabolism of proteins and/or malfunctioning of liver (Harper et al., 1977). The principal cells of the epididymal epithelium synthesize proteins which are androgen dependent (Killian et al., 1973).

Glycogen is the polymer of glucose and is known as animal starch in muscle. Glycogen is the main metabolic fuel in the muscle tissue of majority of animals (Wittenberger, 1996). Glucose is the most readily available source of energy in the animal tissue (Stetten, 1956; Mayer, 1977). Glycogen is an important reserve for the production of glucose. Sertoli cells and spermatogonia often contain glycogen and so may serve as a source of glucose for the tubular cells (Cavazos and Melamby, 1954). In rat testis, glucose is an essential substrate for the maintenance of tissue integrity, for maximal oxygen uptakes, ATP production and protein synthesis (Means and Hall, 1968). Glucose, the major source of energy in the spermatocytes and spermatids is metabolized via the Embden-Meyerhof pathway of glycolysis, acetyl CoA formation and citric acid cycle. The biochemical changes often ensure very early in response to toxicants and following the progressive development of biochemical lesions as a function of dose and length of exposure to toxicants.

In the present graded dose exposure study, the mice treated with 2, 3 and 4 mg methomyl treatment showed significant decrease in the levels of protein and glycogen in the testis and epididymis, whereas the epididymal protein and glycogen was not changed with 2 mg methomyl treatment. However, the mice treated with 1 mg methomyl showed no significant change in the level of protein and glycogen in testis and epididymis. Durational exposure study with 4 mg methomyl treatment for 10, 20 and 30 days showed significant decrease in the levels of protein and glycogen in testis and epididymis, except the epididymal protein and glycogen was not changed in 10 days of methomyl treatment. However, exposure of methomyl for 5 days showed no significant change in the levels of protein and glycogen.
protein and glycogen. Similar observations have been made in rats and mice exposed to mancozeb (Shivanandappa and Krishnakumari, 1990; Kacker et al., 1997; Ksheerasagar and Kaliwal, 2003, 2010), carbosulfan (Ksheerasagar, 2005), monocrotophos (Ratnasooriya et al., 1996), methyl parathion (Prashanti et al., 2006), heachlorohexane (Sanjay et al., 1998), phosphomidon (Shreelaxmi, 2007) and Indoxacarb (Mudaraddi, 2009).

In the present study, it has been found that increasing dose and prolong exposure to methomyl caused decrease in the levels of protein and glycogen. The observed results in the levels of protein and glycogen in testis and epididymis may be due to genotoxic action of methomyl (Uphouse et al., 1984; Bohra and Bhunya, 1987; Saxena et al., 1997) or effect on hormones which are essential for the regulation of DNA and RNA synthesis which in turn influence protein synthesis (Hamilton et al., 1968; Lockard et al, 1982) or lower content of protein and glycogen content was possibly due to direct effect of methomyl on protein and glycogen metabolism or due to enhanced proteolytic activity as a consequence of increased metabolic demands following exposure to the toxic stress or increased catabolism to meet the energy demand of the animal under stress induced by pesticide (Ivanova-Chemishanska, 1982). Thus the effect was reflected by testicular damage and nucleic acid synthesis in albino mice.

Cholesterol

Most of the cholesterol is derived from lipoproteins or from high density proteins. Free cholesterol is insoluble incytosol and is transferent into mitochondria by sterol carrier protein. Once it has reached the mitochondria cholesterol can be cleaved into pregnenolone by cytochrome P<sub>450</sub> SCC (side chain cleavage). Further successive steps continued for steroidogenesis. Cholesterol functions as a precursor molecule during the synthesis of steroid hormones (Preidkalns and Wober, 1968) and also for vitamin D, which is essential for the regulation of calcium and phosphorous metabolism and bone growth. The amount of fat present in any tissue is the net effect of deposition and mobilization. The fat deposited in various tissues may be derived from either dietary fat or de novo synthesis. Both synthesis and mobilization are processes that are self-regulating to some extent but many hormones influence the rate of lipid metabolism and thus there is a balance between lipogenesis and lipolysis. Free cholesterol is insoluble in cytosol and is transported into mitochondria. In this way cholesterol functions as precursor for steroid hormone synthesis, cholesterol is also essential for membrane lipids synthesis. Inhibition in the activity of enzymes involved in cholesterol breakup results into deposition of cholesterol into the cell. The increase in
testicular concentration of total cholesterol level is almost an index of reduced steroidogenesis (Dorfman et al., 1963). The desensitisation of steroidogenesis is associated with depletion of cholesterol in Leydig cells (Freeman and Ascholi, 1981). The increase in the level of cholesterol in testis and high sudanophilic lipid accumulation in the interstitial tissue of drug treated mice supports the inhibition of androgen production which may be due to inadequate availability of LH which is essential to carry out steroidogenesis in the Leydig cells (Hansson et al., 1973). It has been reported that mild hyperlipidimia induced by cholesterol feeding to male rats caused significant reduction in sperm density in cauda epididymis and testes (Purohit and Daradka, 1999). The change in cholesterol level is considered to be very important as it plays a vital role in spermatogenesis (Dorfman et al., 1967).

In the present graded dose exposure study, the mice treated with 2, 3 and 4 mg methomyl showed significant increase in the level of cholesterol in testis and epididymis, except the epididymal cholesterol was not changed with 2 mg methomyl treatment. However, treatment with 1 mg methomyl caused no significant change in the cholesterol level. The durational exposure study with an effective dose 4 mg methomyl for 10, 20 and 30 days showed significant increase in the level of cholesterol in testis and epididymis, except the epididymal cholesterol not changed with 10 days of methomyl treatment. However, treatment with methomyl for 5 days showed no significant change in the cholesterol level in the testis and epididymis. Similar results have been observed in rats and mice treated with monocrotophos, cythion, tamaron, mancozeb, carbosulfan, phosphomidon and indoxacarb (Hanabhy et al., 1991; Adilaxamamma et al., 1995; Ksheerasagar and Kaliwal, 2003, 2010; Ksheerasagar, 2005; Shreelaxmi, 2007; Mudaraddi, 2009).

The results of the present study also indicated that increase in dose and prolong exposure of methomyl caused an increase in cholesterol level of the testis and epididymis which might be due to inhibition of steroidogenesis in testes and adrenal (Shivanandappa and Sarkar, 1979; Shivanandappa and Krishnakumari, 1981; Chitra et al., 1987; Dorfman et al., 1997; Sujatha et al., 2001). Joshi et al (2005) have reported that mancozeb treated rats (500 mg/kg b.w./day for 30 days) caused significant increase in the testicular cholesterol deposition and also significant decrease in testosterone level and concluded that mancozeb exerts toxic effect on male spermatogenesis confirmed by decreased androgen concentration (Bedwal et al., 1994). In present study there is significant decrease in the
testosterone level on methomyl treatment. Further, Inhibition of Cyt P450 enzyme (which is essential for cholesterol break up) in the gonads of rats following administration of monocrotophos an organophosphate pesticide has been reported by earlier workers (Siddiqui et al., 1987). Therefore, in the present study the reduced activity of the important enzyme Cyt P450 thus causes deposition of cholesterol in the cell. Thus, this might have caused reduced steroidogenesis (Eik-Nes and Hall, 1962; Dorfman et al., 1963; Hansson et al., 1973; Shivanandappa et al., 1981), which plays a vital role in spermatogenesis in the testis (Dorfman et al., 1967) indicating effect on production of testicular androgen and other physiological and histological observations. Further, the accumulation of cholesterol (Braze, 1976) in the testes is a direct evidence of antiandrogenic action (Murugravel and Akbarsha, 1991). Since cholesterol being an important precursor in the synthesis of steroid hormones (Turner and Bagnara, 1978) its requirement for normal activities of the testes has been well established.

**Sialic acid**

The sialic acid found at the end of the oligosacharide chains of many soluble glycoproteins will continue to circulate in the blood stream or be removed by the liver. The half-life of LH and FSH are influenced by their oligosaccharides terminated by sulfate and sialic acid respectively. Human LH and FSH have sialic acid as 35% and 83% respectively. The higher content of sialic acid in human FSH contributes to it's half-life being longer than that of LH (Peckham et al., 1973; Baenziger et al., 1988). Sialic acid is concerned with the stabilization of the plasma membrane, maintenance of sperms in a decapitated state, ionic balance in the epididymal plasma and antigen interaction between sperm and epididymal epithelium (Rair et al., 1973; Rajalakshmi et al., 1976). The synthesis and/ or secretion of sialic acid is under androgenic control (Brooks et al., 1974; Bohmer et al., 1977). Sialic acid is a carbohydrate component attached with protein to form glycoproteins. Sialic acid is important secretory productions of the epididymal epithelium is presumably bound to proteins and occur as sialoproteins (Rajalakshmi and Prasad, 1968). These sialoproteins in the epididymis may play a role (i) in the stabilization of the plasma membrane and the acrosome and its membranes (ii) in maintaining spermatozoa in a decapitated state. (iii) in maintaining the ionic balance in the epididymal plasma (Prasad et al., 1973; Rajalakshmi et al., 1976). The Sialic acid is required for sperm maturation in the epididymis (Bose et al., 1974; Gupta et al., 1974b).
In the present graded dose exposure study, the mice treated with 2, 3 and 4 mg methomyl showed significant decrease in the level of sialic acid in testis and epididymis, except the epididymal sialic acid not changed with 2 mg methomyl treatment. However, 1 mg methomyl showed no significant change in the level of sialic acid in testis and epididymis. The durational exposure study with an effective dose 4 mg methomyl for 10, 20 and 30 days showed significant decrease in sialic acid level in testis and epididymis, except the epididymal sialic acid not changed with 10 days of methomyl treatment. However, treatment of methomyl with an effective dose 4 mg for 5 days showed no significant change in the level of sialic acid in testis and epididymis. Similar results have been observed in mice treated with carbosulfan and phosphomidon, (Ksheerasagar, 2005; Shreelaxmi, 2007). It has also been reported that rats treated with endosulfan, caused decrease in sialic acid content and concluded inhibition of spermatogenesis (Choudhary and Joshi, 2003). Cooper et al (1985) described that peroxide attack on glycoproteins occurs largely at the histidine residues, with simultaneous peptidolysis. They also suggested that the mechanism most probably involves the liberation of hydroxyl radical by the Cu+/Cu2+ dependent oxidation-reduction cycle. Most of the sialic acid is abundant as the terminal sugar of sialoglycoprotein and sialoglycolipids in vivo. Further, a report indicated that the glycosidic linkage of sialic acid is a potential target for superoxide and other related ROS (Eguchi et al., 2005). Levinsky et al., 1983 have observed decrease in the levels of sialic acid and also necrotic condition of the testis in rats. Kackar et al., (1997) have reported that mancozeb administered at doses of 500, 1,000 and 1,500 mg/kg body weight/day for 30, 90, 180 and 360 days in rats revealed decrease in sialic acid and protein content of testis and epididymis and serum cholesterol level was increased in dose dependent manner in mancozeb treated rats. Joshi et al (2005) have reported that mancozeb treated rats (500 mg/kg b.wt./day for 30 days) caused significant decrease in the testicular sialic acid and significant decrease in the level of testosterone and concluded that mancozeb exerts toxic effect on male spermatogenesis. Reduction in testicular sialic acid content may be due to absence of spermatozoa or reduced androgen production (Dixit and Gupta, 1987). In the present study the observed alteration in the sialic acid level in reproductive tissues indicates decrease in the levels of gonadotropic hormones, FSH and LH and other glycoproteins thereby affecting normal functioning of gonads and accessory reproductive organs.
d) Effect of methomyl on Lactate dehydrogenase (LDH) and Succinic dehydrogenase (SDH) activity in the testis and epididymis

LDH is involved in glucose metabolism and is widely distributed in all organs of the body. Chemically induced stress causes elevated LDH activity. LDH is commonly used marker enzyme for lethal cell injury (Lock et al., 1993; Lash et al., 1995; Valentovic and Ball, 1998). Most of the cells contain LDH and when these cells are lethally injured, loss of membrane integrity can be determined by monitoring activity of LDH. The LDH release is commonly used as a marker for necrotic/ osmotic cell death (Lash et al., 1995; Valentovic and Ball, 1998). Its increased level is reported in liver necrosis and many other diseases (Varley, 1980). The elevated LDH activity is a marker of tissue damage and serves as a good diagnostic tool in toxicology. A very high value of LDH is severe liver necrosis following exposure to carbon tetrachloride has been reported (Berman et al., 1992). An increase in cerebrospinal fluid LDH activity has been reported in the presence of tumours in central nervous system (Varley, 1980). The methyl parathion treated rats showed an enhanced level of serum and liver LDH activity (Dikshith et al., 1991). It has been suggested that carbosulfan treated mice caused elevated activity of LDH and decrease in the activity of SDH in ovary, testis, liver and kidney (ksheersagar and Kaliwal, 2006).

SDH is one of the key mitochondrial, an iron-containing flavoprotein enzyme and its activity reflects the metabolic activity of any tissue (Padykula, 1952). This enzyme is bound to inner surface of the inner mitochondrial membrane. This mitochondrial enzyme is associated with late stages of spermatogenesis. Administration of testosterone results in enhancement of cellular respiratory rate, increase in mitochondrial number and succinic dehydrogenase activity. The change in SDH activity reflects impairment in TCA cycle. SDH activity is associated with pachytene stage of spermatocytes and increases markedly with the maturation of germ cells (Hodgen and Sherins, 1973) and is distributed throughout the seminiferous epithelium (Turpianen et al., 1962).

In the present graded and durational exposure study, the findings revealed that the activity of LDH is increased and SDH activity decreased significantly in the testis and epididymis of mice treated with 3 and 4 mg methomyl. However, with 1 mg and 2 mg methomyl treatment caused no significant change in the activity of LDH and SDH in the testis and epididymis. The durational exposure study with 4 mg methomyl for 20 and 30
days caused significant increase in the activity of LDH and decrease in the activity of SDH in the testis and epididymis. However, treatment for 5 and 10 days showed no significant change in the activity of LDH and SDH in the testis and epididymis. Similar findings have been reported in rats and mice treated with different pesticides (Dikshith et al., 1991; Pant et al., 1995; Kacker et al., 1997; Mishra et al., 1998; Pant and Srivastava, 2003; Kuladip Jana et al., 2006). Mahgoub and El-Medany (2001) have reported that a carbamate pesticide methomyl caused decrease in testicular succinic dehydrogenase activity.

Qaisar and Setty (1977) have reported estradiol administration increased the total LDH activity in accessory sex organs. The decrease in accessory reproductive organs weight indicates decrease in androgen levels. Similarly, a decrease in androgen level has been suggested for an increased LDH levels in the ventral prostate after thyroidectomy (Vanithakumari and Govindarajulu, 1980). Carbofuran caused alteration in rat testicular dehydrogenase enzyme activities such as sorbitol dehydrogenase (SDH), G-6PD, LDH and γ-glutamyl transpeptidase (Pant et al., 1995). It has also been reported that organophosphate pesticide quinolphos and carbamate pesticides thiram and mancozeb known to cause decrease SDH activity and increased LDH activity in testis of rats (Mishra et al., 1993; Kacker et al., 1997; Pant and Srivastava, 2003). It has been reported that the activities of LDH and SDH in testicular tissue are associated with the maturation of the germinal epithelial layer of seminiferous tubule (Srivastava et al., 1990; Pant et al., 2004). The activity of SDH is increased markedly throughout the maturation of germ cells and is reported to decrease during the depletion of germ cells (Srivastava et al., 1990).

Srivastava and Vijayan (1996) have reported that the activity of SDH enzyme is regulated by testosterone and the diminution of this enzyme activity after arsenic exposure is due to the decreased production of testosterone. LDH is reported to be present in higher amounts in the testis of new born rats and its activity declines with the development of the testis. Testicular and ovarian hormones are known to regulate the activity of LDH and SDH in the uterus and testis (Goodfriend and Kaplan, 1964; Richards and Hiff, 1972; Clark et al., 1973; Srivastava and Vijayan, 1996). All these findings clearly highlight the toxicological significance of LDH and SDH measurements.

Mahgoub and El-Medany (2000) have also reported that the rat treated with methomyl orally (17 mg/kg in saline) daily for two months caused a significant decrease in the level of testosterone was observed in the intoxicated animals, while the levels of FSH,
LH and prolactin were significantly increased. Testicular succinic dehydrogenase enzyme activity was significantly reduced. Further, histopathological studies of the intoxicated rat testis revealed variable degrees of degenerative changes in the seminiferous tubules up to total cellular destruction and they concluded that subchronic exposure to methomyl insecticide has an obvious deleterious effect on rat testes. Thus, the rise in the activity of LDH and reduced activity of SDH in the present study may be due to (a) Effect of pesticide on carbohydrate metabolism in the tissue is indicated by decrease in SDH activity (Guraya, 1985; Preidkalns and Weber, 1968) as this enzyme is related with high metabolic activity such as absorption and secretion (Padykula, 1952). The rise in LDH activity in tissue suggested high turnover of pyruvate to lactate and vice-versa to yield required energy to overcome pesticide induced metabolic stress (Kacker et al., 1997; Mishra et al., 1998; Pant and Srivastava, 2003). (b) Impaired steroidogenesis as these enzymes are regulated by testosterone (Srivastava and Vijayan, 1996; Kuladip Jana et al., 2006). (c) Deterioration of germinal epithelium of seminiferous tubule and depletion in the number of spermatogenic cells (Srivastava et al., 1990; Srivastava and Vijayan, 1996; Pant et al., 2004; Kuladip Jana et al., 2006).

e) Effect of methomyl on 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) activity in testis

3β-HSD and 17β-HSD plays a key role, as these are the prime enzymes in testicular androgenesis (Ghosh et al., 1990; Jana and Samanta, 2006). Both the enzymes are localized in the microsomal fraction of Leydig cells. Activities of these two enzymes are less in immature rat testis and increased in the mature testis (Inano et al., 1970). 3β-HSD is an microsomal NAD-dependent enzyme that catalyses the conversion of pregnenalone to progesterone, 17α-hydroxy pregnenalone to 17α-hydroxy progesterone, dehydro epiandrosterone to androstenedione, androstenedione to testosterone (Hedin et al., 1987; Sasano et al., 1989; Darrel et al., 1995; Simpson et al., 1994). 17β-HSD is an essential enzyme leading to the biosynthesis of sex-steroids since they catalyse the final steps in testosterone (t) and estrogen (E2) steroid hormones. 3β-HSD and 17β-HSD are the rate limiting enzymes in the biosynthesis of steroid hormones (Setchell, 1978). The first step in steroid hormone synthesis is the conversion of cholesterol to pregnenalone in mitochondria is catalysed by P450SCC (side chain cleavage) enzyme (Lanbeth et al., 1985; Zlotkin et al., 1986) which catalyses the conversion of androgen to estrogen (Staffel-Wagner, 2001). The carbamate pesticide Diethyl dithiocarbamate and organophosphorous pesticides
monocrotophos is known to inhibit cyt-450 (Sidiqui et al., 1987; Slotta et al., 1997). The rate of steroidogenesis is dependent upon the generation of NADPH for phosphogluconate pathway, in which G-6PD occupies the position (Savard et al., 1965). G-6PD is potential generator of NADPH, which is required for hydroxylation reaction of steroid biosynthesis (Hall, 1970), whereas, there are reports that the carbamate pesticide carbofuran, carbendazim in rat testis (Duaz et al., 2003; Rajeswary et al., 2007) and diethylthiocarbamate in various tissues of goldfish (Lushchak et al., 2003) decreased the level of G-6PD activity. The Leydig cells and sertoli cells of the seminiferous tubules are known to be the principal sites of steroid hormone biosynthesis as they possess these important steroidogenic enzymes (Blackshaw, 1970).

The results of the present study revealed that the treatment of graded dose and durational exposure of methomyl with 2, 3 and 4 mg showed significant decrease in 3β-HSD and 17β-HSD activities, except 3β-HSD was not changed in 2 mg methomyl treatment in the testis. However, treatment with 1 mg methomyl showed no significant change in 3β-HSD and 17β-HSD activities in the testis. In durational exposure study with an effective dose of 4 mg methomyl for 10, 20 and 30 days showed significant decrease in the activity of 3β-HSD and 17β-HSD in the testis. However, methomyl treatment for 5 days showed no significant change in the activity of 3β-HSD and 17β-HSD in the testis. Similar findings have been reported in rats treated with endosulfan for 30 days inhibited 3β-HSD activity and steroidogenesis (Chitra, 1999). Shivanandappa et al., (1981) have reported inhibition of 3β-HSD activity in adrenal cortex of rats fed with BHC. It has also been found that in rats fed with hexachlore cyclohexane caused inhibition of 17β-HSD and 3β-HSD and G-6PD in the testis as revealed by histochemical studies (Shivanandappa and Krishnakumari, 1983; Shivanandappa and Krishnakumari, 1982). Similarly, a organochlorine insecticide endosulfan, sodium aresenite, lithium chloride and lindane in rats shown to induce testicular androgenesis by inhibiting 3β-HSD and 17β-HSD activity by affecting steroidogenesis (Shaw et al., 1979; Kerr and Sharpe, 1986; Ghosh et al., 1990; Chitra, 1999; Sujata et al., 2001; Sarkar et al., 2003; Jana and Samata, 2005; Kuladip Jana et al., 2006). Rejeswary et al., (2007) have reported that rats treated with carbamate fungicide carbofuran (25 mg/kg bd.wt orally) for 48 days caused significant reduction in testis weight, serum testosterone and estradiol, in addition to this, Leydig cellular activities of steroidogenic enzymes such as 3β-HSD, 17β-HSD, antioxidant enzymes SOD, CAT, GPx, GR, GST, gamma-GT, G-6-PDH were also diminished and suggests that chronic low dose
treatment of carbendazim is capable of inducing reproductive toxicity through increased oxidative stress.

Therefore, the reduced activities of the important enzymes of steroidogenesis observed in the present study employ that toxic action of methomyl was exerted by impaired steroidogenesis in the Leydig cells and Sertoli cells of the seminiferous tubules (Blackshaw et al., 1970; Shivanandappa and Krishnakumari, 1990) or may be due to inhibition of certain pathways of steroidogenesis (Shivanandappa et al., 1981; Chitra et al., 1999; Sujatha et al., 2001; Sarkar et al., 2003; Jana et al., 2005; Kuladip, 2006) by affecting cytochrome P450 and G-6PD (Sidiqui et al., 1987; Stott et al., 1997; Siva Prasada and Ramana, 1978; Reddy et al., 1988) or may be a result of low levels of FSH and LH as these two gonadotropins are prime regulator of testicular androgenic enzymes activities (Shaw et al., 1979; Kerr and Sharpe, 1986; Stott et al., 1997) or might be due to high affinity of binding of pesticide (Zarh et al., 2002) or may be due to pesticide caused oxidative stress (Rejeswary et al., 2007) causing testicular toxicity either by its direct action on reproductive system or by indirect action through neuroendocrine system as reflected by impaired spermatogenic disorder by the diminution in the number of spermatogenic cells and physiological observations.

f) Effect of methomyl on sodium potassium adenosine triphosphatase (Na\(^+\)-K\(^+\)ATPase), Magnesium adenosine triphosphatase (Mg\(^++\)ATPase) and Calcium adenosine triphosphatase (Ca\(^++\)ATPase) activity in the testis and epididymis

ATPases are membrane bound enzymes concerned with immediate release of energy and are responsible for large part of basic metabolic and physiological activities. They have a vital role in the release and uptake of the biogenic amines in (CNS) (Banks, 1965; Paton et al., 1971) and in nutrient transport (Lockall and Pfeffer, 1982). ATPases activity can be taken as meaningful index of cellular activity and forms a useful toxicological tool. ATPase enzyme is associated with lipoprotein in the form of complex (Nakao et al., 1974). Na\(^+\)-K\(^+\)ATPase is also linked to the active transport of sugars and various amino acids including several neurotransmitters (Slotkin et al., 1978; Desaiyah, 1984). Both estrogen and progesterone exhibit significant inhibitory actions on the Na\(^+\)-K\(^+\)ATPase pump in brain and in number of other tissues as well (Davis et al., 1978; La Bella et al., 1985; Rodriguez del Castello et al., 1987; Fraser and Sarnacki, 1989; Fraser and Swanson, 1994). Testosterone is known to stimulate the Na\(^+\)-K\(^+\)ATPase pump in many tissues including the brain (Guerra et al., 1987; Fraser and Swanson, 1994). ATPase serves
as important indicators of any change in male reproduction. Castration in rats causes a decrease in the specific activity of Na\textsuperscript{+}-K\textsuperscript{+}-dependent ATPase in the prostate and seminal vesicles and these changes are prevented by androgen replacement in these castrated animals (Fransworth, 1968). Further, addition of Mg\textsuperscript{2+} and either K\textsuperscript{+} and Na\textsuperscript{+} ions alone to prostatic microsomal membrane preparations obtained from castrated adult rats have restored the Na\textsuperscript{+}-K\textsuperscript{+} ATPase activity (Ahmed and Williams-Ashman, 1969). The adenosine triphosphatases play an important role in the ionic transport that accompanies the secretory activities of the seminal vesicles (Brandes, 1974; Garbers and Kopf, 1980). Testosterone has profound influence on the activities of ATPase in the seminal vesicle and prostate of rats (Stafford et al., 1949; Kumarani, 1981). The epithelial membrane of the prostate and seminal vesicles of rats were found to be rich source of ATPases. The ATPases are involved in the ionic transport associated with secretory activity (Nalbandov, 1976). The ATPases have differential distribution pattern among sub cellular fractions of the seminal vesicles of monkeys. While Na\textsuperscript{+}, K\textsuperscript{+}, Mg\textsuperscript{2+} dependent ATPases were more in the number and mitochondrial fractions, Ca\textsuperscript{2+} dependent ATPases was found to be high in mitochondrial fraction alone. This may be due to the importance of these enzymes in different metabolic activities involving different sub cellular organelles and membrane transport (Arunakaran et al., 1985).

The results of the present investigation with graded and prolong exposure of methomyl study revealed that, the activity of Na\textsuperscript{+}-K\textsuperscript{+}ATPase, Mg\textsuperscript{2+}ATPase and Ca\textsuperscript{2+}ATPase were decreased significantly in the testis and epididymis in the mice treated with 3 and 4 mg methomyl, except Ca\textsuperscript{2+}ATPase activity was not changed significantly with 3 mg methomyl treatment in testis and epididymis. However, with 1 and 2 mg methomyl treatment showed no significant change in the activity of ATPases. The durational study with an effective dose 4 mg methomyl for 20 and 30 days showed decrease in the activity of Na\textsuperscript{+}-K\textsuperscript{+}ATPase, Mg\textsuperscript{2+}ATPase and Ca\textsuperscript{2+}ATPase in testis and epididymis. However, treatment of methomyl for 5 and 10 days showed no significant change in the activity of ATPases in the testis and epididymis, the activity of Na\textsuperscript{+}-K\textsuperscript{+}ATPase decreased significantly in the testis treated with 10 days of methomyl exposure. Similar findings have been reported in rats treated with organophosphate pesticides such as methyl parathion, parathion and phosphomidon (Basha and Nayeeunmussna, 1993; Blasiak, 1995; Shreelaxmi 2007). It has been reported that carbamate pesticide thiram, carbosulfan inhibited Na\textsuperscript{+}-K\textsuperscript{+}ATPase in rat testis (Mishra et al., 1998; Ksheersagar, 2005).
It has been reported that the environmental xenobiotics specially pesticides are known to have a strong affinity for interaction with membrane lipids (Antunes-Maderia and Maderia, 1987). Cell membrane is believed to be the site of action of insecticides by altering structural and functional integrity of cell membrane and also affects Na⁺-K⁺ ATPase and Mg⁺⁺ATPase (Shaw et al., 1995; Rauchova et al., 1995). Mg⁺⁺ATPase is involved in ATP synthesis through oxidative phosphorylation in mitochondria (Boyer et al., 1977). Pesticides exert biologic effect on ATPase system by partitioning in the enzyme complex (Kinter et al., 1972), which may cause allosteric change that result in decreased ATPase activity (Reddy et al., 1992).

Calcium ions play a major role as an important intra cellular messenger in many tissues particularly in central nervous system. It is also involved in various synaptic functions like neurotransmitter release and turnover, generation of Ca²⁺ spikes and regulation of Ca²⁺ dependent K⁺ channels (Moorthy et al., 1987). Active transport by sarcoplasmic reticulum plays a pivotal role in muscle contraction (Kodavanti et al., 1990). These enzymes are well known targets of organochlorine and organophosphorous pesticide compounds (Brown and Sharma, 1976; Pala et al., 1991). It has been found that mevinphos inhibited Na⁺-K⁺ATPase and Mg⁺⁺ATPase in chicken spinal cord (Brown and Sharma, 1976), DDT malathion, ethyl and methyl parathion decreased renal total ATPase and Na⁺-K⁺ATPase in rat (Riedel and Christenson, 1979; Jarmillo-Jurez, 1989; Pala et al., 1991).

In the present study, therefore, the reduced activity of Na⁺-K⁺ATPase, Mg⁺⁺ATPase and Ca⁺⁺ATPase in testis and epididymis may be due to (a) Mitochondrial disorganization as reported during pesticidal toxicosis (Pardini et al., 1980) might have caused inhibition of ATPases in testis and epididymis. (b) Pesticide induced effect on cell membrane because of their strong affinity for interaction with number of lipids (Antuner-Maderia and Maderia, 1987) causing inhibition of membrane bound ATPase enzymes by affecting enzyme complex (Kinter et al., 1972; Basha and Nayeemunnisa, 1993; Shaw et al., 1995; Ruchova et al., 1995; Blaisak, 1995; Mishra et al., 1998). (c) Inhibition of testicular androgenesis as testosterone is known to stimulate the Na⁺-K⁺ATPase pump in many tissues including brain (Guerra et al., 1987; Fraser and Swanson, 1994) which lead to impaired gonadal function.

**g) Effect of methomyl on acid phosphatase (ACP) and alkaline phosphatase (AKP) activity in testis and epididymis**
Phosphatases catalyse the hydrolytic cleavage of phosphoric acid esters. They are designed either “acid” or “alkaline” phosphatases according to their pH optima. Alkaline phosphatases occur in practically in all animals and human tissues. Phosphatases are generally located on absorptive or secretory surface of cells as membrane bound enzymes. Acid phosphatases (ACP) which hydrolysis the ester linkage of phosphate esters at acidic pH (between 5 to 6) and helps in autolysis of the degenerated cells (De Duve et al., 1955). Alkaline phosphatases (AKP), which splits phosphorous esters at alkaline pH (10) and mediates membrane transport is intimately associated in protein synthesis (Pilo et al., 1972), Secretory activity (Ibrahim et al., 1974) and glycogen metabolism (Gupta and Rao, 1974). The pH is one of the main factor which influences many aspects of cell structure and function. Biological control of pH of cells and body fluids is therefore, of central importance in all aspects of metabolism and cellular activities. Both ACP and AKP are commonly found in most tissues of the body like bone, serum, liver and kidney etc. Lysosomes are sub cellular entities that perform important physiological functions through intracellular digestion and phagocytosis in most eukaryotic cell system (Novikoff, 1961; De Duve and Wallianx, 1966) including those of female and male reproductive tract (Doff, 1969; Nagoha et al., 1989; Dikshith et al., 1991). Alkaline phosphatase is known to be responsible for the transference of metabolites across the plasma membrane (Elizabeth and Conell, 1972). It has also associated with the tissue growth differentiation and secretory activity (Malone, 1960). Acid phosphatase is one which helps in autolysis of the degenerated cells (De Duve et al., 1955). AKP which splits phosphorous esters at alkaline pH and mediates membrane transport and is also intimately associated in protein synthesis (Pilo et al., 1972), synthesis of certain enzymes (Summer, 1965), secretory activity (Ibrahim et al., 1974) and glycogen metabolism (Gupta and Rao, 1974) and it has been implicated in a number of important cellular processes such as transport of material across plasma membrane of secretory cells (Goldfischer et al., 1964), phospholipid and nucleic acid synthesis (Gavasto and Pileri, 1958).

Alkaline and Acid phosphatase have vital role in male reproduction. During the sexual development of the rat, the level of acid phosphatase (ACP) increased concurrently with the maturation of spermatocytes (Vanha-Perttula and Nikkanen, 1973). ACP was suggested as a marker enzyme for testis. The activity of ACP is dependent on spermatogenic stage and maturation in testis (Blackshaw, 1973). It has been localized in the lysosomes of Sertoli cells and in golgi vacuoles and in spermatogonia, spermatocytes and spermatids in the testis of the rats (Tice and Bannett, 1963) and mice (Dieteri, 1966; Porier,
ACP and AKP are mainly regulated by androgens in the seminal ventral prostate (Melamby and Cavzos, 1953). ACP activity is androgen dependent and its activity has been shown to rise and fall with the levels of androgen (Stafford et al., 1949). Alkaline phosphatase is the characteristic enzyme of male accessory sex organs as a whole and its distribution differs from that of acid phosphatase. ACP and AKP are sensitive functional indicators of the reproductive status of the animal (Gosh et al., 1990) and AKP is associated with transport of metabolites across the cell membranes (Rackallio, 1970). Both the enzymes serve as markers for androgen action in the target organ (Mann, 1964; Niemi and Kormano, 1965; Mann et al., 1981).

The results of the present investigation revealed that the activity of ACP decreased significantly and AKP activity was increased significantly in testis and epididymis of mice treated with 3 and 4 mg methomyl treatment. However, 1 and 2 mg methomyl treatment caused no significant change in testis and epididymis ACP and AKP activities. The durational exposure study with 4 mg for 20 and 30 days showed significant decrease in ACP activity and increase in AKP activity in testis and epididymis. However, 5 and 10 days exposure to methomyl showed no significant change in ACP and AKP activity in testis and epididymis. Similar changes in the activities of ACP and AKP have been reported in endosulfan and velthane treated teleosts (Venkateshwarulu et al., 1990), carbon tetrachloride treated rats (Berman et al., 1992), HCH treated mice (Ravinder et al., 1989) and carbosulfan treated mice (Ksheerasagar and Kaliwal, 2006). It has been reported that carbamate pesticides such as thiram, mancozeb and carbosulfan exposure caused decrease in ACP and increase in AKP activities in testis of rats (Kacker et al., 1997; Mishra et al., 1998, Ksheerasagar, 2005). Increased levels of AKP activity was also reported in methyl benzimidial carbamate treated rats (Janardhan et al., 1987) and dichlorovos treated rats (Srivastava et al., 1989). Decreased and increased activity of ACP and AKP in liver, testis and serum were also reported by HCH, methyl parathion, diazinon, monocrotophos, phosphamidon, sodium arsenite, indoxacarb treated rats and mice (Dikshith et al., 1975; Janardhan and Sisodia, 1990; Bhatnagar and Soni, 1990; Dikshith et al., 1991; Prashanti et al., 2006; Kuladip Jana et al., 2006; Narayana et al., 2006, Shreelaxmi, 2007; Mudaraddi and Kaliwal, 2009a).

Thus, the reduced activity of ACP and rise in the activity of AKP in the present study may be due to (a) absorptive or secretory surface of the cell membrane causing cell damage hence by reducing the activity of ACP and elevated AKP activity as an adaptive
rise to the persistent stress (Murphy and Porter, 1966; Janardhana et al., 1987; Kacker et al., 1997; Mishra et al., 1996; Abraham and Wilfred, 2000; Pant and Srivastava, 2003). (b) Testicular degeneration, which may likely be a consequence of suppressed testosterone and indicative of lytic activity (Novikoff, 1961; Tice and Bemett, 1963; Dieter, 1966; Riar et al., 1973; Porter, 1975; Kaur et al., 1999). (c) Inhibition of testicular androgenesis as these enzymes are androgen dependent (Stafford et al., 1949; Melamby and Cavzos, 1953; Gosh et al., 1990).

h) Effect of methomyl on oxidative stress parameters in the testis of mice.

Oxidative stress has been defined as an imbalance between the prooxidant/antioxidant steady state in the cell, with the excess of prooxidants being available to interact with cellular macromolecules to cause damage to the cell, often resulting in cell death. Although the occurrence of reactive oxygen species in normal metabolism and the concept of oxidative stress was derived from these studies, it is apparent that oxidative stress can occur in almost any tissue, producing a variety of deleterious effects (El-Sayed et al., 2006). To date, a number of liver diseases, including alcoholic liver disease, metal storage diseases, and cholestasis liver disease, have been shown to have an oxidative stress component (Halliwell and Gutteridge, 1998). Reactive oxygen and reactive nitrogen radicals can be formed in a number of ways the former primarily as a by-product of mitochondrial electron transport. Superoxide, hydrogen peroxide, singlet oxygen, and hydroxyl all arise from this source (Droge, 2002). Other sources include monooxygenases and peroxisomes. If not detoxified, reactive oxygen species can interact with biological macromolecules such as DNA and protein or with lipids. Once lipid peroxidation of unsaturated fatty acids in phospholipids is initiated, it is propagated in such a way as to have a major damaging effect on cellular membranes.

Depression of antioxidants, such as reduced glutathione (GSH), ascorbic acid, ubiquinone, and vitamin E, is often used as evidence of oxidative stress (Scott and Eaton, 1997), and although measuring them is not very difficult, reduced levels of these substances is not a perfect predictor of oxidative stress because they also have a prooxidant action (Scott and Eaton, 1997). Changes in the activity of several antioxidant enzymes have also been used as indirect measures of oxidative stress (Scott and Eaton, 1997), for example, induction of specific enzymes, such as glutathione s-transferase, glutathione peroxidase,
superoxide dismutase, and catalase is often measured in humans as the result of oxidative stress (Scott and Eaton, 1997).

Oxidative stress (OS) has been identified as one of the factor that affects fertility status and thus, has been extensively studied in recent years. Spermatozoa, like any other aerobic cell, are constantly facing the “oxygen-paradox” (Sies, 1993). Oxygen is essential to sustain life as physiological levels of reactive oxygen species (ROS) are necessary to maintain normal cell function. Indeed, breakdown products of oxygen such as ROS can be detrimental to cell function and survival (Lamirande and Gagnon, 1995). Reactive oxygen species are present as free radicals. Examples of ROS include the hydroxyl ion, superoxide, hydrogen peroxide, peroxyl radical, and hypochlorite ion. These are the common forms of ROS that have been considered to be injurious to sperm survival and function when present in abundance (Makker et al., 2009).

ROS is exclusively considered to be toxic to human spermatozoa. However, substantial evidence suggests that small amounts of ROS are necessary for spermatozoa to acquire fertilizing capabilities (Gagnon et al., 1991; Aitken, 1997; Aitken, 1999). Low levels of ROS have been shown to be essential for fertilization, acrosome reaction, hyperactivation, motility, and capacitation (Griveau and Lannou, 1997; Agarwal et al., 2004). Capacitation has been shown to occur in the female genital tract, a process carried out to prepare the spermatozoa for interaction with the oocyte. During this process, the levels of intracellular calcium, ROS, and tyrosine kinase all increase, leading to an increase in cyclic adenosine monophosphate (cAMP). This increase in cAMP facilitates hyperactivation of spermatozoa, a condition in which they are highly motile (Aitken, 1995; Visconti et al., 1995). However, only capacitated spermatozoa exhibit hyperactivated motility and undergo a physiological acrosome reaction, thereby acquiring the ability to fertilize (de Lamirande and Gagnon, 1997). Co-incubation of spermatozoa with low concentrations of hydrogen peroxide has been shown to stimulate sperm capacitation, hyperactivation, acrosome reaction, and oocyte fusion (Aitken, 1995; Aitken, 1997; de Lamirande and Gagnon, 1993; Kodama et al., 1996). Other ROS such as nitric oxide and superoxide anion are shown to promote capacitation and acrosome reaction (Griveau et al., 1995). ROS also have been implicated in sperm-oocyte interaction (Agarwal et al., 2007). Lipid peroxidation caused by low levels of ROS leads to modification of the plasma membrane, thus facilitating sperm-oocyte adhesion (Kodama et al., 1996). Indeed, a
comprehensive review has listed published data on over 600 chemicals to which humans can be exposed that affect the male reproductive system (Krause, 2008).

Reports on toxic effect of male reproductive system indicated that chemicals can potentially damage spermatogenesis at any stage from proliferating spermatogonia to mature spermatozoa. Excess production by abnormal sperm cytoplasmic remnants or seminal leukocytes can cause oxidative damage (Tremellen, 2008). Diploid germ cells may be more vulnerable than their haploid equivalents to spontaneous cell death (Kerr, 1992) and possibly also to apoptosis induced by testicular toxicants. Broadly, three different and not mutually exclusive toxic effects are possible that are cell death, sub-lethal cell damage or genetic change. Lethally damaged cells either die within the epithelium or are shed into the lumen of the seminiferous tubule. Those dying \textit{in situ} may do so by necrosis i.e an uncontrolled lysis and non-specific spilling of cellular contents, which may cause a local inflammatory reaction, or by apoptosis, a physiological process of programmed cell death whereby a doomed cell breaks down into smaller, apoptotic bodies that are then phagocytosed by sertoli cells without bystander damage (Kerr \textit{et al.}, 1972).

Reports indicated that carbamate pesticides such as maneb, disulfiram, N-N-diethylthiocarbamate, aldicarb, propoxur, thiram, carbaryl and sodium methylthiocarbamate induces oxidative stress in rats and mice (Vioque-Fernandez \textit{et al.}, 2009; Pruett \textit{et al.}, 2009; Ahmed \textit{et al.}, 2010; Grosicka-Maciag \textit{et al.}, 2010; Valentine \textit{et al.}, 2010; Maran \textit{et al.}, 2010; Rana and Shivanandappa, 2010). Endosulfan, an organochlorine pesticide from the group of cyclodienes, influences antioxidative enzymes in heart and, therefore heart injury associated with this insecticide may be due to oxidative tissue damage. While Vitamin E was inhibiting formation of free radical, also it decreased endosulfan cardiotoxicity (Jalili \textit{et al.}, 2007). Further, oxidative stress, generated by xenobiotics, induces disturbances in antioxidant enzyme systems (Gabbianelli \textit{et al.}, 2002). Free radicals play an important role in the toxicity of pesticides and environmental chemicals. Pesticide chemicals may induce oxidative stress leading to generation of free radicals and alteration in antioxidant or oxygen free radical scavenging enzyme system (Banerjee \textit{et al.}, 1999). The effects of organophosphate insecticides on fish revealed that besides acetylcholinesterase inhibition, there were changes characteristic of oxidative stress (Malkovics \textit{et al.}, 1995). In humans, pesticides were shown to reduce the total cholesterol and phospholipids level of RBC membrane following phosphamidon and malathion, and increase lipid peroxides level following malathion (John \textit{et al.}, 2001).
Glutathione (GSH) and Ascorbic acid

Glutathione is a tripeptide composed of three different amino acids: glutamate, cysteine and glycine that has numerous important functions within cells. Glutathione contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain. Glutathione is a nucleophilic scavenger and an electron donor via the sulfhydryl group of its business residue, cysteine. Glutathione (GSH) plays a fundamental role in the antioxidant biology of mammals. Severe GSH depletion is associated with pathologic consequences including, but not limited to, susceptibility to the development of lipid peroxidation (Gillette et al., 1974; Mitchell and Jollows, 1975; Anundi et al., 1979). Glutathione is widely distributed tripeptide and found mainly in the cell cytosol (Kosower and Kosower, 1978). This plays a crucial role in the detoxification process (Mitchell, 1976). The rat and mouse testis have been reported to contain high concentrations of GSH content (Calvin, and Turner, 1982). The GSH pools in the cytosol were also observed in testis, suggesting the protective role of GSH and related enzymes (Thimmappa and Muralidhara, 2007). GSH is the major cellular sulfhydryl compound that serves as an effective reductant and a nucleophile that interacts with numerous electrophilic and oxidizing compounds.

Ascorbic acid is an important micronutrient necessary for a significant number of metabolic reactions in humans and other primates (Frei et al., 1989). Ascorbic acid deficiency is characterized by increased oxidative stress and tissue injury resulting in inhibition of ascorbic acid biosynthesis (Barja et al., 1994; Brown et al., 1997; Maeda et al., 2000). Antioxidant such as ascorbic acid, vitamin E and GSH protect germ cells against oxidative DNA damage and play an important role in spermatogenesis (Fraga et al., 1991). Ascorbic acid is a major chain-breaking antioxidant and is present in the extracellular fluid. It neutralizes hydroxyl, superoxide, and hydrogen peroxide radicals and prevents sperm agglutination (Agarwal et al., 2004). In fact, deficiency of ascorbic acid and vitamin E causes the disturbance of spermatogenesis (Chinoy et al., 1986; Bensousas et al., 1998). Ascorbic acid is a potent water-soluble antioxidant in biological fluids, scavenging physiologically relevant reactive oxygen and nitrogen species, thereby preventing oxidative damage to vital biomolecules (Thyagaraju and Muralidhara, 2008). Ascorbic acid is a potent antioxidant known to protect tissues from oxidative injury (Frei, 1991, 1999). Vitamin C is a potent antioxidant known to protect tissues from oxidative injury (Frei et al., 1991, 1999). Loading cells with vitamin C reduces oxidative cell death (Guaiquil et al., 2004).
2001; Witenberg et al., 1999), inhibits FAS-induced apoptosis (Perez-Cruz, et al., 2003), and confers genomic protection (Lutsenko et al., 2002) through the quenching of intracellular ROS. Vitamin C is a cofactor to enzymes involved in the synthesis of collagen (Padh et al., 1991) and carnitine (Rebouche et al., 1991), and is postulated to be involved in the mitochondrial reduction of α-tocopherol (Packer, 1979) and ferri-cytochrome c (Myer et al., 1980). In specialized cells, vitamin C is directly transported as ascorbic acid (AA) via sodium-dependent vitamin C transporters (SVCT) (Tsukaguchi et al., 1999; Daruwala, et al., 1999). However, most cells transport vitamin C in its oxidized form, dehydroascorbic acid (DHA), via facilitative glucose transporters (Glut), including Glut1 (Vera et al., 1993). Once inside cells, DHA is reduced and accumulated as ascorbic acid (AA) (Vera et al., 1994).

In the present graded dose exposure study, there was a significant decrease in the levels of GSH and ascorbic acid in the testis of mice treated with 2, 3 and 4 mg methomyl except, ascorbic acid where it was not changed significantly in the testis with 2 mg methomyl treatment. However, treatment with 1 mg methomyl showed no significant change in the levels of GSH and ascorbic acid. The durational exposure study for 10, 20 and 30 days treatment with an effective dose 4 mg methomyl revealed a significant decrease in the levels of GSH and ascorbic acid in the testis, except ascorbic acid where it was not changed significantly in the testis with 10 days of methomyl treatment. However, treatment with 4 mg methomyl for 5 days caused no significant change in both the levels of GSH and ascorbic acid in the testis.

Similar results have been reported that damage to testicular male germ cells induced by various xenobiotics, products of abnormal metabolism, or ROS can result in testicular dysfunction, leading to infertility (Aitken et al., 1994, 1995; Stohs and Bagchi, 1995; Sikka et al., 2001). In this regard, the development of a hydroperoxides model of oxidative stress (OS) in testis and examined the correlation among lipid peroxidation (LPO) in testis, DNA damage, and genotoxic implications (Rajeshkumar and Muralidhara, 1999; Rajeshkumar et al., 2002). It has also been confirmed that germ cells are more susceptible to oxidative stress than somatic cells (Zhang et al., 2006). It has been shown that the pyrethroid insecticide, cypermethrin, significantly induced free radical production in plasma, liver, brain and testes (el-Demerdash et al., 2003). Recently, Ozden and Alpertunga, (2010) have reported that carbamate pesticide methiocarb treated rat revealed increased lipid peroxidation and decreased GSH in liver, kidney, brain, and testis and they concluded that
methiocarb-induced toxicity may be associated with oxidative stress to cellular membranes. Similarly, Abarikwu et al., (2010) have reported that atrazine treated rats at high dose (120 and 200 mg/kg body weight orally for 7 and 16 days) caused increased lipid peroxidation, decreased GSH, GST, SOD and CAT activities in testis and epididymis and they also observed dose dependent adverse effect on the testicular and epididymal sperm numbers, motility, viability, morphology, and daily sperm production and suggested that atrazine impairs reproductive function and elicits a depletion of the antioxidant defense system in the testis and epididymis, indicating the induction of oxidative stress.

Di (2-ethylhexyl) phthalate DEHP-induced apoptosis in the testis was observed primarily with pachytene spermatocytes, but not with sertoli cells. Because sertoli cells contain higher concentrations of GSH, vitamin E and GSH-related enzymes than pachytene spermatocytes and round spermatids, differences in their susceptibility to phthalate toxicity may be explained by the difference in their activities of antioxidants and related enzymes. DEHP decreased testis levels of free thiol, GSH and ascorbic acid in a dose-dependent manner (Yoganathan et al., 1989; Bauche et al., 1994). Increased myocardial TBARS and depletion of GSH provide evidence for oxidative stress due to oral administration of lindane apart from the liver, oxidative stress has also been demonstrated in the testes (Chitra et al., 2001). Many of the metabolites of lindane are also conjugated with glutathione, causing depletion of the glutathione reserve (Roy Ananya et al., 2005) this may be the reason for the decrease in glutathione levels in the study. It has been shown that the different treatments have generated oxidative stress due to intoxication of diethyl maleate (DEM) and Tertbutyl hydroperoxide (TBHP) (Sumiti and Bansal, 2009), diethyl maleate (DEM) is a thiol alkylating agent (Friedrichs et al., 1998), reacts with the thiol group of glutathione (GSH) and hence decreases its concentration within the cell (Ghosh et al., 2005). Reduction in GSH results in the increase of free radicals as the free radicals produced during normal metabolic processes are not scavenged by GSH. Decreased GSH is unable to match its potential to scavenge ROS which are normally generated in the tissues (Haidara et al., 1999). Tertbutyl hydroperoxide (TBHP) on the other hand is ROS generating cytotoxin (Cheng et al., 2007) and increases ROS concentration by producing different free radicals (mainly superoxide anion) directly in the cells (Haidara et al., 2002). The adverse effects of methoxychlor on the male reproductive system have been described, consisting in by decreasing the antioxidants and enzymes in the epididymal sperm of goats (Gangadharan, 2001) and rats (Koner, 1998;Latchoumycandane, 2002). It has been reported that methyl parathion (MP) is known to induce abnormalities in sperm shape and
decrease the sperm count in rodents, possibly mechanism related to reduced ascorbic acid level in the testis (Mattew et al., 1992; Narayana et al., 2005a). These facts indicate that the defense mechanism against oxidative stress plays critical roles in the maintenance of spermatogenesis and prevention of testicular atrophy. It has been reported that depletion of GSH in lung and kidney may in part contribute to the decrease in ascorbic acid observed following 0.15 ng TCDD /kg /day exposure (Slezak et al., 2000). Reduced ascorbic acid level due to intoxication of di (2-ethylhexyl) phthalate (DEHP) in germ cells has also been shown (Emiko et al., 2002).

Thimmappa and Muralidhara (2007) have reported that oxidative Stress induced by organic Hydroperoxide (OH) in testis and epididymal sperm of rats caused increased LPO and protein carbonyl levels in testis and epididymal sperm, depletion of glutathione and ascorbic acid levels in testis, and suggested that depletion of GSH levels in testis can explain a decreased concentration of ascorbic acid, which enters the cells mainly in oxidized form, where it is reduced by GSH. The diminution of ascorbic acid levels has serious implications, since, in addition to its antioxidant function, it also participates in the regeneration of other antioxidants. Significant decrease in ascorbic acid, GSH and tocopherol and clearly suggests that testis is indeed subjected to significant oxidative stress (Ong et al., 2002).

Ascorbic acid is possibly indicating its role as a potential scavenger of ROS. On the contrary, ascorbic acid has long been established as an agent to play a crucial role in the differentiation process of the spermatogonial cells to sperm (Steinberger and Steinberger 1966). Consequently its use as an antioxidant to fight against increased oxidative radicals, eventual insufficiency of the vitamin may occur in the mice testes (Acharya et al., 2003), possibly failing to participate appropriately in the array of differentiation programme leading to transformation of sperm, thereby resulting in a significant decline in the sperm count. Furthermore, ascorbic acid deficiency is characterized by increased oxidative stress and tissue injury resulting in the inhibition of ascorbic acid biosynthesis (Barja et al., 1994; Brown et al., 1997; Maeda et al., 2000). Antioxidant such as ascorbic acid, vitamin E and GSH protect germ cells against oxidative DNA damage and play an important role in spermatogenesis (Fraga et al., 1991; Krakowska et al., 2006). There are many reports where supplement of ascorbic acid suppressed oxidative damage in male reproductive organs (Farombi et al., 2008; Ei-Seweidy et al., 2008; Mehta and Singh, 2009; Botelho et al., 2009; Aydogan et al., 2010; Farias et al., 2010).
In the present study, the decreased levels of GSH and ascorbic acid in the testis of mice under the influence of methomyl treatment may be due to a) Increased oxidative stress as methomyl is sulphur containing N-methyl carbamate compound prone to bind various antioxidants and anti-oxidation enzymes (Banerjee et al., 1999; El-Khawaga., 2005) or (b) Metabolites of pesticides may conjugate with glutathione, causing depletion of the glutathione reserve (Roy Ananya et al., 2005) or (c) Accumulation of lipophilic components or conjugates of pesticides and metabolites produce oxidative stress and these will increase the concentration of MDA or protein carbonyl may be the reasons in the present study.

Thiobarbaturic acid (TBARS) and protein carbonyl

Lipid peroxidation (LPO) is the most extensively studied manifestation of oxygen activation in biology. LPO is broadly defined as “oxidative deterioration of poly unsaturated fatty acids (PUFA)” which is fatty acids that contain more than two carbon carbon double bonds (Halliwell, 1990). Lipid peroxidation (LPO) is a chain reaction between polyunsaturated fatty acids and ROS, and it produces lipid peroxides and hydrocarbon polymers that are both highly toxic to the cell. Malonyldialdehyde (MDA) is an end product of peroxidation of polyunsaturated fatty acids and related esters, and is, therefore, used as a marker of lipid peroxidation. Besides other adverse effects, ROS also induce lipoperoxidation that changes membrane permeability; it leads to protein impairment, and to enzyme inactivation and at the end to DNA damage (Rao and Shaha, 2000). Plasmatic membranes of spermatozoa contain high concentrations of polyunsaturated fatty acids and therefore, are highly sensitive to oxidative stress (Halliwell and Gutteridge, 2000).

Protein carbonyls are employed as useful biomarkers of ROS mediated protein oxidation (Levine et al., 1990) and elevated levels of oxidized proteins in animal tissues under various oxidative stress situations are documented (Grune et al., 2004). ROS can lead to oxidation of amino acid residue side chains, formation of protein-protein cross linkage and oxidation of protein backbone resulting in protein fragmentation. Further, protein carbonyls are introduced into the protein by reaction with aldehydes such as MDA and 4-hydroxy-nonenal (4-HNE), which are the end products of lipid peroxidation (Droge, 2002). Among the various oxidative modifications of amino acids in proteins, protein carbonyl
formation may be an early biomarker of ROS-mediated protein oxidation (Ong et al., 2002).

In the present graded dose exposure study, there was a significant increase in the levels of TBARS and protein carbonyl in the testis of mice treated with 3 and 4 mg methomyl. However, treatment with 1 and 2 mg methomyl showed no significant change in the levels of TBARS and protein carbonyl. The durational exposure study for 10, 20 and 30 days treatment with an effective dose 4 mg methomyl revealed a significant increase in the levels of TBARS and protein carbonyl in the testis. However, treatment with 4 mg methomyl for 5 days caused no significant change in the levels of TBARS and protein carbonyl in the testis. Similar results were obtained by Mudaraddi and Kaliwal (2009a) on administration of Indoxacarb in albino mice.

It is reported that lindane causes oxidative stress in the testes and increases lipid peroxidation (Chitra et al., 2001). It has been reported that chlorpyrifos, paraquat and diquat causes oxidative stress and causes the increase in MDA in different tissues of rats (Osman, 1999; Salama, et al., 2001). Debnath and Mandal, (2000) have reported that testicular damage caused by quinalphos was due to free radical-mediated LPO. Abdollahi et al., (2004) have reported effects of malathion to oxidative stress and AchE activity in saliva and plasma in rats following subchronic exposure. Studies have results shown that malathion at doses of 100, 500, and 1500 ppm increased plasma thiobarbituric acid reactive substances by 61, 69, and 63%. Yarsan et al., (1999) have reported that aldicarb and malathion causes lipid peroxidation, indicating increased plasma MDA content in rodents. In another study of Yarsan et al., (2002) the effects of deltamethrin on lipid peroxidation for subacute, subchronic, and chronic periods were investigated. Their results indicated that MDA levels increased especially for the subchronic and chronic periods in rats. Seth et al., (2000) have studied the effects of propoxur on lipid peroxidation. In that study they found that propoxur increased MDA levels and altered the glutathione levels in rats. In another study chlorpyrifos-induced oxidative stress and caused tissue damage in the liver, kidney, brain and fetus in pregnant rats, where there was increase in LPO, significant increase in TBARS, decrease in level of GSH and SOD in plasma and tissues and also caused significant fetal deaths (Zama et al., 2007). Methomyl is also known to cause LPO in liver, kidney and erythrocytes (El-Khawaga., 2005; Mansour et al., 2009).
Among the various oxidative modifications of amino acids in proteins, protein carbonyl formation may be an early biomarker of ROS-mediated protein oxidation (Ong et al., 2002). ROS are known to modify or inactivate proteins in a variety of pathways (Fagan et al., 1999; Choy et al., 2008). Generally, ROS may cause reversible and/or irreversible modifications on sensitive proteins (Fagan et al., 1999). Reversible modifications, usually at cysteine residues, with dual role of protection from irreversible damage and modulation of protein function (Choy et al., 2008). Irreversible modifications induced by ROS such as carbonyl formation and accumulation of protein carbonyl by-products in tissues are generally associated with permanent loss of protein function, and are considered as indicator of severe oxidative damage and disease-derived from protein dysfunction (Fagan et al., 1999). ROS can react directly with the protein by oxidizing amino acid residue side-chains into ketone or aldehyde derivatives or they can react with molecules such as sugars and lipids, generating reactive carbonyl species that then can react with protein (Stadtman and Berlett et al., 1998; Zusterzeel et al., 2000).

Zimmerman et al., (2004) have reported that carbamate pesticide thiocarbamate can form reactive sulfoxide and sulfone intermediates, which may be involved in the toxicity of thiocarbamates through covalent modification of cysteine and serine active sites of enzymes forming S-(N, N-Dialkylaminocarbonyl) cysteine adducts in rat brain, liver, and testes. It has been reported that exposure of dieldrin induces oxidative damage in the mouse striatum, resulted in a 53% decrease in total glutathione, an increase in the redox potential of glutathione, and a 90% increase in protein carbonyls (Hatcher et al., 2007). In another study, the effects of intraperitoneal injection of carbamate pesticide diethylthiocarbamate at a concentration of 0.01 mg/g wet for 48 hrs in Carassius auratus caused significant decrease in activities of SOD, GST and increased CAT activity with significant increase in protein carbonyl levels in the brain, liver and kidney (Lushchak et al., 2007).

In the present study the reason for increased MDA level in testes under the influence of methomyl treatment in mice might caused due to the conjugation of methomyl or its metabolites to the polyunsaturated fatty acids or by production of ROS reacts with polyunsaturated fatty acids or accumulation of lipophilic components of pesticides conjugated with the fatty acids.
Organic hydrogen peroxide (HP) such as tert-butyl hydroperoxide (tbHP) have been employed as a prototypic inducer of oxidative stress in a variety of in vitro (Sakida et al., 1991) and in vivo systems (Younes and Weiss, 1990; Kaur et al., 2006; Li et al., 2006). Evidence of HP-induced oxidative stress in testis was discernible in terms of depletion of reduced GSH/nonenzymic antioxidant molecules, perturbations in the activities of antioxidant enzymes, and higher protein carbonyls (Thimmappa and Muralidhara, 2007). Further, HP-induced oxidative damage in testis was evident from the elevated levels of protein carbonyls at higher doses. Among the various oxidative modifications of amino acids in proteins, protein carbonyl formation may be an early biomarker of reactive oxygen species (ROS)-mediated protein oxidation (Ong et al., 2002). Accumulation of high amounts of carbonyls in testis after 2-week HP exposure reflects a high rate of protein oxidation, consistent with a high degree of LPO in the organ. Further, it also reflects a very low rate of oxidized protein degradation and/or low repair activity, since oxidized forms of some proteins and proteins modified by LPO products not only are resistant to proteolysis but can also inhibit the ability of proteases to degrade the oxidized forms of other proteins (Thimmappa and Muralidhara, 2007).

Issam et al., (2009) have reported that, deltamethrin subcutaneous treatment in male rats caused hypospermatogenesis accompanied by some apoptotic particular cell fragments into the seminiferous tubule, significant decrease in FSH, LH and testosterone and also caused significant increase in malondialdehyde (MDA) and they concluded that deltamethrin treatment causes an arrest of spermatogenesis, a significant disharmony in sex hormones and MDA levels in rats that is related to lipid peroxidation causing oxidative stress which may be one of the molecular mechanism involved in gonadal toxicity. Recently, Abarikwu et al., (2010) have reported that atrazine treated rats at high dose (120 and 200 mg/kg body weight orally for 7 and 16 days) caused increased lipid peroxidation (TBARS), decreased GSH, GST, SOD and CAT activities in testis and epididymis and they also observed dose dependent adverse effect on the testicular and epididymal sperm numbers, motility, viability, morphology, and daily sperm production and suggested that atrazine impairs reproductive function and elicits a depletion of the antioxidant defense system in the testis and epididymis, indicating the induction of oxidative stress.

In the present study, the increased level of TBARS and protein carbonyl in testis of mice under the influence of methomyl treatment may be due to (a) Reactive oxygen species (ROS) generated by pesticide that may damage the proteins themselves (Thyagaraju and
Muralidhara, 2008) or it is supported indirectly by the fact that the protein carbonyl content was significantly enhanced in the testis. Further pesticide may directly interact with the enzymes and inhibit their activities (Thyagaraju and Muralidhara, 2008) and elevated levels of oxidized proteins in animal tissues under various oxidative stress situations are documented (Grune et al., 2004). (b) Similarly ROS can lead to oxidation of amino acid residue side chains, formation of protein-protein cross linkage and oxidation of protein backbone resulting in protein fragmentation. Accumulation of high amounts of carbonyls in testis might be due to exposure reflects a high rate of protein oxidation, consistent with a high degree of LPO in the organ or due to very low rate of oxidized protein degradation and/or low repair activity, since oxidized forms of some proteins and proteins modified by LPO products not only are resistant to proteolysis but can also inhibit the ability of proteases to degrade the oxidized forms of other proteins (Thimmappa and Muralidhara, 2007). (c) Accumulation of lipophilic components of pesticides and metabolites produce oxidative stress and these will increase the concentration of MDA may be one of the reason in the present study.

Super oxide dismutase (SOD) and Catalase (CAT)

Every cell has defence mechanism against the free radicals. The antioxidant enzymes SOD, CAT and GST constitute a mutually supportive team of defense against ROS (Tabatabaie and Floyd, 1994; Bandhopadhy et al., 1999). SOD and CAT are known to play an important role in scavenging ROS. SOD catalyzes the destruction of the superoxide radicals. CAT is known to reduce the H$_2$O$_2$ into water and oxygen to prevent oxidative stress and in maintaining cell homeostasis. Seminal plasma provides a protective environment for spermatozoa by its enzymatic contents including superoxide dismutase, catalase and glutathione peroxidase and non-enzymatic antioxidants such as glutathione, ascorbate, urate, etc. (Alvarez, et al., 1995; Sikka, et al., 1996; Armstrong, et al., 1998; Saleh and Agarwal, 2002), some of which originate in the epididymis (Potts et al., 1999).

High potassium depolarises the membrane, reducing ion transport activities. During their time in the epididymis spermatozoa are likely to be protected from lipid peroxidation by certain secretory products of the epididymis such as superoxide dismutase and glutathione peroxidase (Williamson et al., 1998; Potts et al., 1999) and from damage by leaking acrosomal enzymes by a secreted acrosin inhibitor (Kirchhoff, 2007). These activities have been reported to be an indicator of tissue’s ability to cope with oxidative stress (Husain and Somani, 1997; Mimic-Oka et al., 1999). Thus an increase in SOD and CAT might be in
response to increased oxidative stress. However, when a condition of oxidative stress strongly establishes, the defense capacities against ROS becomes insufficient (Halliwell and Gutteridge, 2000) in turn ROS also affects the antioxidant defense mechanisms, reduces the intracellular concentration of GSH and decreases the activity of SOD and CAT. It has also been known to decrease the detoxification system produced by GST (Yamamoto and Yamashita, 1999). Increasing evidence indicates that oxidative stress causes organ injury and carcinogenesis (Stal and Olson, 2000).

Reactive oxygen species (ROS) must be continuously inactivated to keep only a small amount necessary to maintain normal cell function. Antioxidants, in general, are compounds and reactions, which dispose, scavenge, and suppress the formation of ROS, or oppose their actions. Among the well-known biological antioxidants, SOD and its two isozymes, and catalase have a significant role. SOD spontaneously dismutates (O$_2^-$) anion to form O$_2$ and H$_2$O$_2$, while catalase converts H$_2$O$_2$ to O$_2$ and H$_2$O.

\[
\text{SOD} \quad 2(O_2^-) + 2H \rightarrow H_2O_2 + O_2 \\
\text{Catalase} \quad H_2O_2 \rightarrow H_2O + \frac{1}{2}O_2
\]

There are three biological forms of SOD bearing an important antioxidant activity,

1. Mitochondrial tetrameric manganese-containing Mn-SOD, which is mainly present in mitochondria, but is synthesized in cytosol and coded by a nuclear gene

2. Cytosolic dimeric copper/zinc-containing Cu/Zn-SOD

3. Extracellular SOD - tetrameric glycoprotein containing Cu/Zn which occurs mostly in the extracellular and interstitial space.

In the present graded dose exposure study, there was a significant decrease in the activities of SOD and CAT in the testis of mice treated with 3 and 4 mg methomyl treatment. However, treatment with 1 and 2 mg methomyl showed no significant change in the levels of CAT and SOD. The durational exposure study for 20 and 30 days treatment with an effective dose 4 mg methomyl revealed a significant decrease in the levels of SOD and CAT activities in the testis. However, treatment with 4 mg methomyl for 5 and 10 days caused no significant change in the activities of SOD and CAT in the testis.
Recently, Bansal and Bilaspuri (2010) have reviewed on oxidative stress and antioxidants on semen functions and stated that oxidative stress (OS) has been considered a major contributory factor to the infertility. Oxidative stress is the result of imbalance between the reactive oxygen species (ROS) and antioxidants in the body which can lead to sperm damage, deformity, and eventually male infertility. Although high concentrations of the ROS cause sperm pathology (ATP depletion) leading to insufficient axonemal phosphorylation, lipid peroxidation, and loss of motility and viability. It has been reported that lindane causes oxidative stress in the testes and alters the catalase and SOD activity might be due to higher activities of SOD and catalase following adaptation might have protected the testes from more severe injury due to oxidative stress. It has been reported that due to intoxication of diazinon an increase in testicular superoxide dismutase (SOD) activity was detected on 32 day (Sarabia et al., 2009). Similar changes have also been reported in the epididymis of rats treated with methoxychlor. Gangadharan et al., (2001) have reported that the specific activities of superoxide dismutase, glutathione peroxidase and glutathione reductase decreased in methoxychlor-incubated sperm of goats, while the level of lipid peroxidation was increased in a dose-dependent manner, as compared to the corresponding controls. Similar results were also obtained by Latchoumycandane et al., (2002) in the testes of rats after oral administration of methoxychlor in the daily doses of 50, 100 and 200 mg/kg body weight for 1, 4 and 7 days. It has been reported that carbamate fungicide carbendazim known to diminish activities of SOD, CAT, GST and markedly elevates LPO and ROS in Leydig cells of rats with the administration of 25 mg/kg body weight orally for 48 days (Rajeswary et al., 2007). Recently, Ozden and Alpertunga (2010) reported that carbamate pesticide methiocarb treated rats caused significant increase in the lipid peroxidation (TBARS) with significant decrease in the activities of GSH, SOD, CAT and GSH-Px in testis, liver, kidney, and brain and suggested that methiocarb-induced toxicity may be associated with oxidative stress to cellular membranes (Ozden et al., 2009).

In the present study, decrease in the activities of SOD and CAT in testes of mice under the influence of methomyl treatment may be due to (a) Insufficient scavenging of superoxide and hydrogen peroxide produced due to intoxication of methomyl, where a condition of oxidative stress strongly establishes, the defense capacities against ROS becomes insufficient (Halliwell and Gutteridge, 2000) in turn ROS affects intracellular concentration of GSH and decreases the activity of SOD and CAT (Yamamoto and Yamashita, 1999) (b) Decrease in glutathione and protein-binding sulfhydryl groups, which also causes an increase in the reactive oxygen species like hydrogen peroxide, hydroxyl
radicals, and superoxide radical ions, which in turn, increase lipid peroxidation, thereby resulting in reduced intercellular stability, damage to DNA and cell membranes, and cell death (Stohs, 2001).

**Glutathione-s-transferase (GST)**

GST is an enzyme, which is involved in the detoxification process. An important function of GST in response to oxidative stress is its ability to conjugate GSH with lipid peroxidation products (Rao and Shaha, 2000). They conjugate substrate xenobiotics with utilization of reduced glutathione (GSH); the glutathione conjugates being more water soluble are easily eliminated from the body. In addition to conjugation reactions, some members of the GST superfamily can serve as peroxidases and isomerases (Mannervik and Danielson, 1988). They have been shown to be instrumental in protecting against electrophiles and products of oxidative stress (Hayes et al., 2005). It has been reported that carbamate fungicide carbendazim is known to diminish activities of SOD, CAT, GST and markedly elevates LPO and ROS in Leydig cells of rats with the administration of 25 mg/kg body weight orally for 48 days (Rajeswary et al., 2007). Recently, in another study the carbamate pesticides both aldicarb and propoxur significantly inhibits the activity of GST and increased the level of TBARS in CHO-K1 cells with cell injury due to increased oxidative stress (Maran et al., 2010).

In the present graded dose exposure study, there was a significant decrease in the activities of GST in the testis of mice treated with 2, 3 and 4 mg methomyl treatment. However, treatment with 1 mg methomyl showed no significant change in the level of GST activity. The durational exposure study for 10, 20 and 30 days treatment with an effective dose 4 mg methomyl revealed a significant decrease in the activity of GST in the testis. However, treatment with 4 mg methomyl for 5 days caused no significant change in the activity of GST in the testis.

The biotransformation enzymes participate not only in the metabolism of naturally occurring chemicals, such as secondary plant metabolites and toxins in ingested plants, fungi and animals, but also in the metabolism of various artificial chemicals and drugs (Gregus and Klaasen, 1996). Xenobiotic metabolism is typically divided into phase-I (functionalization) and phase-II (conjugation) reactions. Phase-I enzymes, for example the cytochrome P450 (CYP), catalyse the incorporation of a functional group (-OH, -NH2, -SH or -COOH) into the initially hydrophobic substrate. Phase-II enzymes, for example
glutathione-s-transferases (GST), make the molecule less reactive by conjugation of the functional group with glutathione, sulphate or glucuronic acid. These reactions generally make the substrate water-soluble, and the conjugated endogenous compound further facilitates the excretion of the product (Hayes and Pulford, 1995).

In the present study, GST activity was significantly reduced with high dose and prolonged exposure of methomyl. In agreement with these results, Mansour et al., (2009) have found that methomyl decreased the activity of SOD, GST and increased LPO in rat erythrocytes. According to Garg et al., (2008) acute 24 hr single oral dose of 9 mg/kg of methomyl treatment to rats resulted in a significant increase in the LPO, further GSH levels and the activities of Catalase and GST were found to be significantly decreased following methomyl treatment. Similarly El-Khawaga, (2005) have found that methomyl decreased the activity of SOD, CAT and GST in mice liver, and Salama et al., (2005) reported that methomyl mode of action could be due to the induction of oxidative stress. In the present study the reason for decreased activity of glutathione s-transferase under the influence of methomyl treatment in mice testes may be due to ROS produced by the methomyl or increased lipid peroxidation which affects the intracellular concentration GSH, which in turn affected the activity of GST. (Halliwell and Gutteridge, 2000; Stohs, 2001).

i) **Agarose gel electrophoresis of cellular DNA in the testis of mice on exposure to methomyl.**

Apoptosis and necrosis are two modes of cell death that have unique morphological, biochemical and physiological characteristics. Necrosis is “accidental” cell death. It is a pathological process, which occurs when cells are exposed to a serious physical or chemical insult, such as in hypoxia, ischemia, temperature fluctuations, disruption of membrane structure and exposure to toxins. Necrosis begins with the impairment of the cell’s ability to maintain homeostasis, leading to an influx of water and extracellular ions. Morphological characteristics include loss of membrane integrity and swelling of the cytoplasm, mitochondria and endoplasmic reticulum, which leads to cell rupture. Upon rupture, the contents of the cytosol, including lysosomal enzymes are released into the extracellular fluid. The major biochemical processes of necrosis are the loss of ion homeostasis, digestion of DNA and post-lytic random DNA fragmentation (late event). The physiological significance is that extensive tissue injury can result due to lysosomal enzyme action, inflammatory responses are likely to be triggered and phagocytosis by macrophages.
is increased leading to higher incidences of respiratory burst and ROS generation (Wyllie et al., 1980; Darzynkiewicz et al., 1997; Boehringer, 1998).

The results of the present study revealed that the intact DNA band was observed in the cells of the testis in the control mice. Mice treated with 1, 2, 3 and 4 mg of methomyl for 30 days and effective dose 4 mg/kg methomyl for 5, 10, 20 and 30 days showed a smear of DNA that indicates of the atrophied cells in the testis of mice. Similar results were reported that the occurrence of DNA fragmentation is considered to be a hallmark of apoptotic cell death when it precedes membrane lysis, DNA fragmentation has also been shown to play a significant role in cell necrosis caused by hepatotoxins (Ray et al., 1993; Fukuda et al., 1993) and in necrotic lymphoma cell death induced by Ca$^{2+}$ ionophore or hypoxia in mice (Collins et al., 1992). It has also been suggested that the appearance of DNA fragmentation may sometimes represent a common final pathway for acute cell death by apoptosis as well as by necrosis (Ray et al., 1993; Fukuda et al., 1993). It has been reported that some kinds of cytotoxic chemicals such as dimethylnitrosamine and thioacetamide induce both apoptosis and necrosis in vivo (Ledda-Columbano et al., 1991). It has also been suggested that D-Galactosamine (D-GalN) also induces apoptosis in the liver of rats (Tsutsu et al., 1997; Stachlewitz et al., 1999). The necrotic and apoptotic neuronal cell death have also been reported in vivo after exposure to peroxynitrite, agarose gel electrophoresis showed a smear of DNA due to random cleavage of base pairs during the necrotic process excitotoxic/ free radical insults (Emanuela et al., 1995). Therefore, in the present study the findings revealed that the mice treated with different doses of methomyl and prolonged exposure showed a smear of DNA which indicates necrotic cell death of the testes in mice may that may be due to random cleavage of base pair during the necrotic process (Emanuela et al., 1995).

j) **Effect of methomyl on serum testosterone levels**

Gonadal steroid hormones regulate gamete formation, growth, development, differentiation and functional physiology of the reproductive tract. They also regulate secondary sexual characters, sexual behaviour and mating patterns. Steroid hormones thus ensure effective consummation of reproductive potentials of animals. Steroid hormones produced by the testis are called androgens or male sex hormones. They regulate spermatogenesis and maturation of spermatozoa. They control the growth, development and differentiation of accessory reproductive organs of the male. In the testis both Leydig cells
and Sertoli cells are the primary sites of androgen biosynthesis. Sertoli cells do not have the enzyme system to cleave the side chain of cholesterol and so they cannot utilize cholesterol for androgen synthesis. Instead they use pregnenalone or progesterone produced by the Leydig cells and also synthesizes testosterone. Testosterone is the principal androgen produced in the testis of majority of mammals including humans.

In the present graded exposure study, the mice treated with 2, 3 and 4 mg showed significant decrease in the level of testosterone in serum. However, 1 mg methomyl showed no significant change in serum testosterone level. In the durational exposure study with an effective dose 4 mg methomyl for 10, 20 and 30 days showed significant decrease in serum testosterone level. However, treatment of methomyl with an effective dose 4 mg for 5 days showed no significant change in serum testosterone level. Similar results have been observed in rats treated with different pesticides like methyl parathion, dimethoate, carbofuran, sodium arsenite, sodium fluoride (Salem et al., 1988; Affif et al., 1991; Ghosh et al., 2002; Ryan et al., 2004; Narayana et al., 2006; Kuladip Jana et al., 2006). Numerous studies on pesticide have showed significant changes in testosterone level. In earlier study, Clement (1985) have found reduced testosterone level in serum of mice intoxicated with soman. Rhoum et al., (2001) have demonstrated a decreased level of testosterone in the serum of rats intoxicated with DDT, due to a decrease of testosterone production by the testes. At high concentrations, DDT has also been shown to cause decrease in the testosterone level of flounders (Millis et al., 2001). Mahgoub and EI-Medany (2000) reported that the rat treated methomyl orally (17 mg/kg in saline, i.p) daily for two months caused a significant decrease in the level of testosterone was observed in the intoxicated animals, while the levels of FSH, LH and prolactin were significantly increased. Testicular succinic dehydrogenase enzyme activity was significantly reduced. Further, histopathological studies of the intoxicated rat testis revealed variable degrees of degenerative changes in the seminiferous tubules up to total cellular destruction and they concluded that subchronic exposure to methomyl insecticide has an obvious deleterious effect on rat testes.

Carbamate pesticides are rapidly degraded in the environment, they analogues to organochlorine insecticides, which can bioaccumulate and biomagnify. Carbamates are toxic to male reproductive system (Rajeswary et al, 2007). Carbamate pesticides such as carbofuran, carbaryl, molinate, mancozeb, carbendazim, pyrrolidine dithiocarbamate and Ethylene--bis-dithiocarbamate caused decrease in testosterone in rats and mice (Beard and
Rawlings, 1998; Shrivastava and shrivastava, 1998; Kevlock and cummings, 2005; Joshi et al., 2005; Rajeswary et al., 2007; Ilbey et al., 2009; Mallem et al., 2009). Indeed, interest in the potential adverse reproductive effects of these compounds has heightened in recent years as a result of studies which showed that several organophosphorous compounds including dimethoate, methyl parathion, malathion, dichlorovos, chlorophyrifos and dimethyl methyl phosphate impair fertility, suppress libido, deteriorate semen quality and cause testicular degeneration in rodents following repeated exposure (Krause and Homila, 1974; Krause, 1977; Haas et al., 1983; Dunnick et al., 1984; Sarkar et al., 2000; Suresh et al., 2005; Narayana et al., 2006; Pina-Guzman et al., 2006; Narayana et al., 2006; Prashanti et al., 2006). Investigators have showed that repeated exposure of dimethoate decreases serum testosterone levels, testicular weight, sperm motility and increases the percentage of dead and abnormal sperm in rats and rabbits (Salem et al., 1988; Afifi et al., 1991). Since spermatogenesis and fertility are critically dependent upon the maintenance of adequate levels of testosterone. Although carbamates may reduce serum steroid hormone levels by increasing steroid catabolism and elimination, several studies have demonstrated that these compounds can directly inhibit steroid hormone production (Joshi et al., 2005; Rajeswary et al., 2007; Ilbey et al., 2009; Mallem et al., 2009).

Thus, the reduced serum testosterone concentration observed in the present study may be due to, (a) Direct effect of methomyl on the anterior pituitary or hypothalamus by inhibiting the release of gonadotropins LH and FSH leading to decreased secretion of testosterone (Patashnit et al., 1978; Whorton et al., 1979; Martin et al., 1998). (b) Decrease in the activity of acetylcholinesterase as methomyl is well known established inhibitor of AChE, resulting in alternations in the pituitary gonadotropins which could have influenced on the gonadal function directly through the effect on the pituitary AChE by affecting androgen levels. (c) Methomyl effect on the reduced activities of 3βHSD and 17βHSD which are indicative of reduced steroidogenesis.
SUMMARY AND CONCLUSION

The present study is aimed to elucidate the graded doses and durational exposure of methomyl on accessory reproductive organs, biochemical contents, steroid hormone and histopathology of testis and epididymis.

1. Treatment with 3 and 4 mg methomyl caused significant decrease in the weight of testes and accessory reproductive organs (epididymides, vasa deferentia, seminal vesicles, prostate gland, coagulatory glands and Cowper’s glands). However, treatment with 1 and 2 mg methomyl caused no significant change in the weight of the testes and accessory reproductive organs, except testis and epididymis weight where there was a significant decrease with 2 mg methomyl treatment. Durational study with 4 mg methomyl for 10, 20 and 30 days caused significant decrease in the weight of testes, epididymides, vasa deferentia, seminal vesicles, prostate gland, coagulatory glands and Cowper’s glands, except seminal vesicles weights not changed significantly in the mice exposed to methomyl for 10 days. However, methomyl exposure for 5 days caused no significant change in the weight of the testes and accessory organs.

2. There was a significant decrease in the number and diameter of spermatogenic and Leydig cells with 2, 3 and 4 mg methomyl treatment. However, treatment with 1 mg methomyl showed no significant change in the number and diameter of spermatogenic and Leydig cells. Treatment of mice with 4 mg methomyl for 10, 20 and 30 days caused significant decrease in the number and diameter of spermatogenic cells (spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells), except number of spermatogonia and Leydig cells and diameter of primary spermatocytes was not changed significantly in 10 days methomyl treatment, whereas treatment with 4 mg methomyl for 5 days caused no significant change in the number and diameter of spermatogenic and Leydig cells.

3. Histologic observations of the testis of the mice treated with 1 mg methomyl revealed a decrease in the number of spermatocytes, and interstitial tissue contains clusters of Leydig cells. The testis of the mouse treated with 2, 3 and 4 mg methomyl showed formation of giant cells, loss of spermatogenic cells and lumen with decreased number of sperms and atrophied seminiferous tubules. Histologic observations of the
testis of the mouse treated with 4 mg methomyl for 5 and 10 days showed abnormal spermatogenesis. There was loss of number of spermatocytes, spermtids and sperms in lumen. The tubular spaces are packed with interstitial tissue. The testis of the mouse treated with 4 mg methomyl for 20 and 30 days showed formation of giant cells, vacuoles, and decreased number of spermatogenic cells and lumen with the loss of sperms. Seminiferous tubules are atrophied. Leydig cells are in deformed condition.

4. Mice treated with 2, 3 and 4 mg methomyl caused significant decrease in the level of DNA, RNA, protein and glycogen, whereas cholesterol content was increased significantly, except DNA was not changed significantly in 2 mg methomyl treatment in testis. However, the level of DNA and RNA was not changed significantly in 1 mg methomyl treated mice. Durational study with 4 mg methomyl for 20 and 30 days caused significant decrease in the level of DNA, RNA, protein and glycogen, whereas cholesterol level was increased significantly. However, treatment with 4 mg methomyl for 5 and 10 days caused no significant change in the levels of biochemical contents of testis.

5. Mice treated with 3 and 4 mg methomyl showed significant decrease in the activity of SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase and ACP. However, the activity of the LDH and AKP were increased significantly in the testis. In the mice treated with 1 and 2 mg methomyl caused no significant change in the activity of enzymes, except in 2 mg methomyl treated mice the activity of the 17βHSD decreased significantly. Durational study with 4 mg methomyl for 20 and 30 days showed significant decrease in SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase and ACP whereas the activity of LDH and AKP were increased significantly. In the mice treated with 4 mg methomyl for 5 and 10 days caused no significant change in the activity of dehydrogenase, phosphatase and steroidogenic enzymes in testis, except 17βHSD and Na⁺-K⁺ATPase activity were decreased significantly in the mice exposed to methomyl for 10 days.

6. There was a significant decrease in the levels of GSH and ascorbic acid, whereas levels of TBARS and protein carbonyl increased significantly in the testis of mice treated with 3 and 4 mg methomyl. However, treatment with 1 and 2 mg methomyl showed no significant change in the levels of antioxidants and oxidative products.
The durational exposure study for 10, 20 and 30 days treatment with an effective dose 4 mg methomyl revealed a significant decrease in the levels of GSH and ascorbic acid, whereas levels of TBARS and protein carbonyl increased significantly in the testis, except ascorbic acid where it was not changed significantly in the testis with 10 days methomyl treatment. However, treatment with 4 mg methomyl for 5 days caused no significant change in the levels of antioxidants and oxidative products in the testis.

7. In the present graded dose exposure study, there was a significant decrease in the activities of SOD, CAT and GST in the testis of mice treated with 3 and 4 mg methomyl treatment. However, treatment with 1 and 2 mg methomyl showed no significant change in the activities of oxidative stress enzymes, except GST which decreased significantly in 2 mg methomyl. The durational exposure study for 20 and 30 days treatment with an effective dose of 4 mg methomyl revealed a significant decrease in the activities of SOD, CAT and GST in the testis. However, treatment with 4 mg methomyl for 5 and 10 days caused no significant change in the activities of oxidative enzymes in the testis.

8. Mice treated with 3 and 4 mg methomyl caused significant decrease in the level of epididymal DNA, RNA, protein and glycogen contents, whereas cholesterol level was increased significantly. Treatment with 1 and 2 mg methomyl showed no significant change in the level of biochemical contents. Durational study with 4 mg methomyl for 20 and 30 days caused significant decrease in the level of DNA, RNA, protein and glycogen whereas cholesterol content was increased significantly. However, mice treated with 4 mg methomyl for 5 and 10 days, showed no significant change in the level of biochemical contents.

9. Treatment with 3 and 4 mg methomyl, caused significant decrease in the activity of SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase and ACP in the epididymis, whereas the activity of LDH and AKP were increased significantly. The mice treated with 1 and 2 mg methomyl caused no significant change in the enzymes activity of epididymis. Durational study with 4 mg methomyl for 20 and 30 days the activity of SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP were decreased significantly, whereas the activity of LDH and AKP were increased significantly. In
the mice treated with 4 mg methomyl for 5 and 10 days, there was no significant change in the activity of enzymes of the epididymis.

10. The mice treated with 2, 3 and 4 mg methomyl showed significant decrease in the level of testosterone in serum. However treatment 1 mg methomyl showed no significant change in the serum testosterone level. The durational exposure study with an effective dose 4 mg methomyl for 10, 20 and 30 days showed significant decrease in the serum testosterone level. However, treatment of methomyl with an effective dose of 4 mg for 5 days showed no significant change in serum testosterone level.

11. Mice treated with 1, 2, 3 and 4 mg of methomyl for 30 days and effective dose of 4 mg/kg methomyl for 5, 10, 20 and 30 days showed a smear of DNA in agarose gel electrophoresis.

In conclusion, the present results suggests that methomyl is a reproductive toxicant in males affecting the synthesis of protein, DNA, RNA, glycogen, cholesterol, steroid enzymes, cell membrane, lysosomal enzyme, antioxidant and stress enzyme activities resulting in testicular degeneration causing cell death in the testis. Further, the loss of gonadal macromolecular constituents DNA, RNA and protein may be due to increased lysosomal activity (catabolism) or independent of the reduced mitochondrial and microsomal activities or increased catabolism of the biomolecules to meet the enhanced energy demand of the animals under stress on their reduced function of various biochemical enzymes or increased oxidative stress. It appears, therefore, that methomyl treatment produces degenerative changes in spermatogenic cells and inhibits androgen production acting primarily at the level of hypothalamo-hypophysial-gonadal axis.
EXPLANATION TO PHOTOMICROGRAPHS

Effect on testes of the albino mice on exposure to methomyl

Fig. 1. T.S. of the testis of the control mouse showing different stages of spermatogenesis includes spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids. Lumen of seminiferous tubules filled with sperms. Interstitial tissue contains clusters of Leydig cells.

Fig. 2. T.S. of the testis of the mouse treated with 1 mg/ kg body weight/ day methomyl for 30 days showing decreased number of spermatocytes, spermatids and sperms in lumen. Interstitial tissue contains clusters of Leydig cells.

Fig. 3. T.S. of the testis of the mouse treated with 2 mg/ kg body weight/ day methomyl for 30 days showing decreased number of spermatogenic cells as a result of giant cells, vacuoles formation and seminiferous tubular lumen possess less number of sperms. Leydig cells are in deformed condition.

Fig. 4. T.S. of the testis of the mouse treated with 3 mg/ kg body weight/ day methomyl for 30 days showing formation of giant cells and decreased number of all stages of spermatogenic cells and lumen with loss of sperms. Leydig cells are in deformed condition.

Fig. 5. T.S. of the testis of the mouse treated with 4 mg/ kg body weight/ day methomyl for 30 days showing formation of giant cells, vacuoles and marked reduction in spermatogenic cells. Lumen with tissue debris and clumped sperm fragments. Leydig cells are in deformed condition.

Photographs original exposures at 200X

SG - Spermatogonia
PS - Primary spermatocytes
SS - Secondary spermatocytes
SP - Spermatids
SM - Sperms
Lu - Lumen
ST - Seminiferous tubule
IN - Interstitial tissue
EXPLANATION TO PHOTOMICROGRAPHS

Temporal effect on testes of the albino mice on exposure to methomyl

Fig. 6. T.S. of the testis of the control mouse showing different stages of spermatogenesis includes spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids. Lumen of seminiferous tubules filled with sperms. Interstitial tissue contains clusters of Leydig cells.

Fig. 7. T.S. of the testis of the mouse treated with 4 mg/ kg body weight methomyl for 5 days showing abnormal spermatogenesis. The seminiferous tubules are closely packed. The tubular spaces are packed with interstitial tissue, containing clusters of Leydig cells.

Fig. 8. T.S. of the testis of the mouse treated with 4 mg/ kg body wt methomyl for 10 days showing different stages of spermatogenesis. Lumen of seminiferous tubule consists less sperms. Leydig cells are in deformed condition.

Fig. 9. T.S. of the testis of the mouse treated with 4 mg/ kg body wt methomyl for 20 days showing formation of giant cells resulting decreased number of all stages of spermatogenesis cells and lumen with loss of sperms. Leydig cells are in deformed condition.

Fig. 10. T.S. of the testis of the mouse treated with 4 mg/ kg body weight/ day methomyl for 30 days showing formation of giant cells, vacuoles and marked reduction in spermatogenic cells. Lumen with tissue debris and clumped sperm fragments. Leydig cells are in deformed condition.

Photographs original exposures at 200X

| SG | Spermatogonia |
| PS | Primary spermatocytes |
| SS | Secondary spermatocytes |
| SP | Spermatids |
| SM | Sperms |
| Lu | Lumen |
| ST | Seminiferous tubule |
| IN | Interstitial tissue |
Fig A: Agarose gel of testis DNA of mice on exposure to methomyl
Lane : 1- Control Lane : 2 - 1 mg/ kg
Lane : 3 - 2 mg/ kg Lane : 4 - 3 mg/ kg
Lane : 5 - 4 mg/ kg

Fig B: Agarose gel of testis DNA of mice on exposure to methomyl
Lane : 1- Control Lane : 2 - 4 mg/ kg for 5 days
Lane : 3 - 4 mg/ kg for 10 days Lane : 4 - 4 mg/ kg for 20 days
Lane : 5 - 4 mg/ kg for 30 days
Table 1.1  Effect on weights of testes and accessory reproductive organs in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/d)</th>
<th>Testes</th>
<th>Epididymis</th>
<th>Vasa deferentia</th>
<th>Seminal vesicles</th>
<th>Prostate gland</th>
<th>Coagulatory glands</th>
<th>Cowper's glands</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>745.6 ± 0.92</td>
<td>333.4 ± 0.54</td>
<td>190.9 ± 0.35</td>
<td>803.0 ± 0.47</td>
<td>95.6 ± 0.26</td>
<td>175.4 ± 0.56</td>
<td>155.0 ± 0.62</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>742.8 ± 0.44</td>
<td>331.6 ± 0.43</td>
<td>189.5 ± 0.47</td>
<td>802.6 ± 0.34</td>
<td>94.9 ± 0.23</td>
<td>172.8 ± 0.72</td>
<td>153.7 ± 0.57</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>700.5 ± 0.94*</td>
<td>327.5 ± 0.60*</td>
<td>188.8 ± 0.34*</td>
<td>801.9 ± 0.35</td>
<td>94.0 ± 0.21</td>
<td>171.1 ± 0.70</td>
<td>153.0 ± 0.56*</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>659.7 ± 0.97*</td>
<td>317.5 ± 0.45*</td>
<td>162.0 ± 0.42*</td>
<td>750.5 ± 0.56*</td>
<td>83.9 ± 0.46*</td>
<td>161.3 ± 1.10*</td>
<td>145.9 ± 0.34*</td>
</tr>
<tr>
<td>V</td>
<td>4</td>
<td>630.5 ± 0.74*</td>
<td>299.3 ± 0.58*</td>
<td>158.6 ± 0.43*</td>
<td>710.5 ± 1.82*</td>
<td>77.5 ± 0.34*</td>
<td>154.6±1.02*</td>
<td>139.0 ± 0.33*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 10 animals  
* Significant P < 0.05 compared with control

Table 1.2  Effect on number and diameter of spermatogenic and Leydig cells in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (days)</th>
<th>Spermatogonia</th>
<th>Primary spermatocytes</th>
<th>Secondary spermatocytes</th>
<th>Spermatids</th>
<th>Leydig cells</th>
<th>Diameter (μm) spermatogenic and Leydig cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spermatogonia</td>
<td>Primary spermatocytes</td>
<td>Secondary spermatocytes</td>
<td>Spermatids</td>
<td>Leydig cells</td>
<td>Spermatogonia</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>67.9 ± 0.18</td>
<td>92.1 ± 0.18</td>
<td>100.6 ± 0.34</td>
<td>168.1 ± 0.58</td>
<td>44.4 ± 0.26</td>
<td>8.0 ± 0.21</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>67.2 ± 0.25</td>
<td>91.6 ± 0.26</td>
<td>99.7 ± 0.21</td>
<td>167.1 ± 0.43</td>
<td>43.4 ± 0.27</td>
<td>7.6 ± 0.16</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>64.1 ± 0.18*</td>
<td>91.2 ± 0.27</td>
<td>97.2 ± 0.36*</td>
<td>164.7 ± 0.39*</td>
<td>42.6 ± 0.22</td>
<td>7.3 ± 0.26</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>62.2 ± 0.20*</td>
<td>87.6 ± 0.16*</td>
<td>94.6 ± 0.16*</td>
<td>162.9 ± 0.50*</td>
<td>38.9±1.00*</td>
<td>6.4 ± 0.16*</td>
</tr>
<tr>
<td>V</td>
<td>4</td>
<td>59.4 ± 0.16*</td>
<td>85.6 ± 0.16*</td>
<td>92.4 ± 0.34*</td>
<td>161.3 ± 0.15*</td>
<td>38.3±0.30*</td>
<td>6.1 ± 0.18*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 5 animals  
* Significant P < 0.05 compared with control
### Table 1.3 Effect on biochemical contents of testis in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/d)</th>
<th>DNA</th>
<th>RNA</th>
<th>Protein</th>
<th>Glycogen</th>
<th>Cholesterol</th>
<th>Sialic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>2.51 ± 0.03</td>
<td>4.68 ± 0.02</td>
<td>147.7 ± 0.15</td>
<td>6.52 ± 0.02</td>
<td>9.25 ± 0.02</td>
<td>15.6 ± 0.16</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>2.46 ± 0.02</td>
<td>4.62 ± 0.03</td>
<td>147.1 ± 0.27</td>
<td>6.48 ± 0.03</td>
<td>9.3 ± 0.01</td>
<td>15.1 ± 0.18</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>2.44 ± 0.03</td>
<td>4.58 ± 0.02*</td>
<td>146.4 ± 0.22*</td>
<td>6.43 ± 0.02*</td>
<td>9.42 ± 0.02*</td>
<td>14.8 ± 0.20*</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>2.33 ± 0.02*</td>
<td>4.13 ± 0.03*</td>
<td>140.5 ± 0.26*</td>
<td>6.15 ± 0.02*</td>
<td>9.62 ± 0.01*</td>
<td>13.6 ± 0.22*</td>
</tr>
<tr>
<td>V</td>
<td>4</td>
<td>2.11 ± 0.02*</td>
<td>3.87 ± 0.01*</td>
<td>135.5 ± 0.37*</td>
<td>5.53 ± 0.02*</td>
<td>9.81 ± 0.01*</td>
<td>12.5 ± 0.16*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 5 animals

* Significant P < 0.05 compared with control

### Table 1.4 Effect on testis dehydrogenase and phosphatase enzymes activity in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/d)</th>
<th>LDH a</th>
<th>SDH b</th>
<th>3βHSD c</th>
<th>17βHSD d</th>
<th>Na*-K+ATPase e</th>
<th>Mg**ATPase e</th>
<th>Ca**ATPase e</th>
<th>ACP f</th>
<th>AKP g</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>9.06 ± 0.02</td>
<td>12.20 ± 0.20</td>
<td>0.44 ± 0.01</td>
<td>0.94 ± 0.01</td>
<td>7.45 ± 0.02</td>
<td>9.00 ± 0.04</td>
<td>6.10 ± 0.03</td>
<td>18.80 ± 0.30</td>
<td>14.10 ± 0.18</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>9.10 ± 0.02</td>
<td>12.15 ± 0.24</td>
<td>0.43 ± 0.02</td>
<td>0.94 ± 0.02</td>
<td>7.42 ± 0.01</td>
<td>8.96 ± 0.03</td>
<td>6.08 ± 0.01</td>
<td>18.60 ± 0.24</td>
<td>14.05 ± 0.10</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>9.11 ± 0.01</td>
<td>12.09 ± 0.20</td>
<td>0.41 ± 0.01</td>
<td>0.85 ± 0.01*</td>
<td>7.38 ± 0.03</td>
<td>8.90 ± 0.02</td>
<td>6.07 ± 0.01</td>
<td>18.50 ± 0.42</td>
<td>14.00 ± 0.12</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>10.08 ± 0.01*</td>
<td>11.60 ± 0.22*</td>
<td>0.37 ± 0.0*</td>
<td>0.79 ± 0.0*</td>
<td>7.00 ± 0.04*</td>
<td>7.80 ± 0.01*</td>
<td>6.05 ± 0.01</td>
<td>17.60 ± 0.30*</td>
<td>14.90 ± 0.10*</td>
</tr>
<tr>
<td>V</td>
<td>4</td>
<td>10.30 ± 0.02*</td>
<td>11.15 ± 0.10*</td>
<td>0.32 ± 0.01*</td>
<td>0.72 ± 0.01*</td>
<td>6.42 ± 0.02*</td>
<td>7.10 ± 0.02*</td>
<td>5.42 ± 0.02*</td>
<td>16.20 ± 0.20*</td>
<td>15.50 ± 0.24*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 5 animals

* Significant P ≤ 0.05 compared with control

a μmoles of pyruvate formed / min / g tissue
d μmoles of NADPH converted to NADP / min / g tissue
b μmoles of formazon formed / min / g tissue
e μmoles of inorganic phosphorus formed / min / g tissue
c μmoles of NAD converted to NADH / min / g tissue
f μmoles of P-nitrophenyl formed / min / g tissue
Table 1.5  Effect on oxidative stress parameters of testes in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/d)</th>
<th>Antioxidants</th>
<th>Oxidative stress byproducts</th>
<th>Oxidative stress enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GSH a</td>
<td>Ascorbic acid b</td>
<td>TBARS c</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>9.75 ± 0.06</td>
<td>362 ± 0.14</td>
<td>0.24 ± 0.14</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>9.72 ± 0.10</td>
<td>360 ± 0.17</td>
<td>0.26 ± 0.16</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>9.61 ± 0.04*</td>
<td>356 ± 0.18</td>
<td>0.28 ± 0.08</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>9.58 ± 0.08*</td>
<td>329 ± 0.14*</td>
<td>0.36 ± 0.12*</td>
</tr>
<tr>
<td>V</td>
<td>4</td>
<td>8.72 ± 0.10*</td>
<td>308 ± 0.12*</td>
<td>0.44 ± 0.15*</td>
</tr>
</tbody>
</table>

a umol of GSH/mg protein
b umol of ascorbic acid/gm wet tissue
c nmoles MDA/mg of tissue protein
d nmoles of protein carbonyl/mg protein
e super oxide dismutase (SOD) unit/mg protein
f umol of H$_2$O$_2$/min/mg protein
g umole /min/mg protein

* Significant P ≤ 0.05 compared to control.

Values are mean ± SEM of 10 animals.

Table 1.6  Effect on biochemical contents of epididymis in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/d)</th>
<th>Biochemical contents (µg / mg wet weight of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DNA</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>2.40 ± 0.03</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>2.38 ± 0.02</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>2.35 ± 0.02</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>2.34 ± 0.01*</td>
</tr>
<tr>
<td>V</td>
<td>4</td>
<td>2.10 ± 0.01*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 5 animals

* Significant P ≤ 0.05 compared with control
Table 1.7  Effect on epididymis dehydrogenase and phosphatase enzymes activity in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/day)</th>
<th>LDH(^a)</th>
<th>SDH(^b)</th>
<th>Na(^+)-K(^+) ATPase(^c)</th>
<th>Mg(^++) ATPase(^c)</th>
<th>Ca(^++) ATPase(^c)</th>
<th>ACP(^d)</th>
<th>AKP(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>15.20 ± 0.18</td>
<td>14.12 ± 0.22</td>
<td>8.62 ± 0.15</td>
<td>10.70 ± 0.51</td>
<td>6.70 ± 0.10</td>
<td>15.40 ± 0.22</td>
<td>13.60 ± 0.40</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>15.16 ± 0.24</td>
<td>14.08 ± 0.16</td>
<td>8.55 ± 0.28</td>
<td>10.62 ± 0.44</td>
<td>6.65 ± 0.24</td>
<td>15.30 ± 0.16</td>
<td>13.66 ± 0.16</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>15.12 ± 0.30</td>
<td>13.98 ± 0.10</td>
<td>8.45 ± 0.30</td>
<td>10.54 ± 0.45</td>
<td>6.62 ± 0.30</td>
<td>15.26 ± 0.28</td>
<td>13.94 ± 0.26</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>16.00 ± 0.20*</td>
<td>13.10 ± 0.34*</td>
<td>7.56 ± 0.30*</td>
<td>9.76 ± 0.24*</td>
<td>6.56 ± 0.38</td>
<td>14.65 ± 0.20*</td>
<td>14.80 ± 0.12*</td>
</tr>
<tr>
<td>V</td>
<td>4</td>
<td>16.70 ± 0.36*</td>
<td>12.84 ± 0.42*</td>
<td>7.10 ± 0.12*</td>
<td>9.51 ± 0.21*</td>
<td>5.96 ± 0.26*</td>
<td>14.32 ± 0.24*</td>
<td>15.36 ± 0.20*</td>
</tr>
</tbody>
</table>

\(a\) μmoles of pyruvate formed/ min/ g tissue  
\(b\) μmoles of formazan formed/ min/ g tissue  
\(c\) μmoles of NAD converted to NADH/min/ g tissue  
\(d\) μmoles of inorganic phosphorus formed/ min/ g tissue  
\(e\) μmoles of P-nitrophenyl formed/ min/ g tissue  

Values are mean ± SEM of 5 animals  
* Significant P ≤ 0.05 compared with control

Table 1.8  Effect on serum testosterone level in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>Testosterone level (ng/ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>52.40 ± 0.45</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>51.30 ± 0.53</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>48.64 ± 0.20</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>32.78 ± 0.30*</td>
</tr>
<tr>
<td>V</td>
<td>4</td>
<td>20.62 ± 0.44*</td>
</tr>
</tbody>
</table>

Values are mean ± SE  
* Significant P ≤ 0.05 compared to control
Table 1.9 Temporal effects on weights of testes and accessory reproductive organs in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (days)</th>
<th>Testes</th>
<th>Epididymis</th>
<th>Vasa deferentia</th>
<th>Seminal vesicles</th>
<th>Prostate gland</th>
<th>Coagulatory glands</th>
<th>Cowper's glands</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>745.6 ± 0.92</td>
<td>333.4 ± 0.54</td>
<td>190.9 ± 0.35</td>
<td>803.0 ± 0.47</td>
<td>95.6 ± 0.26</td>
<td>175.4 ± 0.56</td>
<td>155.0 ± 0.62</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>743.7 ± 0.57</td>
<td>338.0 ± 0.45</td>
<td>189.8 ± 0.45</td>
<td>802.0 ± 0.47</td>
<td>95.0 ± 0.21</td>
<td>173.0 ± 0.68</td>
<td>154.0 ± 0.63</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>700.9 ± 0.80*</td>
<td>324.2 ± 0.67*</td>
<td>180.9 ± 0.36*</td>
<td>801.7 ± 0.33</td>
<td>94.2 ± 0.20*</td>
<td>169.8 ± 0.78*</td>
<td>150.3 ± 0.50*</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>659.7 ± 0.86*</td>
<td>308.5 ± 0.54*</td>
<td>162.3 ± 0.52*</td>
<td>751.8 ± 0.84*</td>
<td>85.2 ± 0.51*</td>
<td>161.7 ± 0.88*</td>
<td>146.3 ± 0.40*</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>630.5 ± 0.74*</td>
<td>299.3 ± 0.58*</td>
<td>158.6 ± 0.43*</td>
<td>710.5 ± 1.82*</td>
<td>77.5 ± 0.34*</td>
<td>154.6 ± 1.02*</td>
<td>139.0 ± 0.33*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 10 animals

* Significant P ≤ 0.05 compared with control

Table 1.10 Temporal effects on number and diameter of spermatogenic and Leydig cells in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (days)</th>
<th>Spermatogonia</th>
<th>Primary spermatocytes</th>
<th>Secondary spermatocytes</th>
<th>Spermatids</th>
<th>Leydig cells</th>
<th>Spermatogonia</th>
<th>Primary spermatocytes</th>
<th>Secondary spermatocytes</th>
<th>Spermatids</th>
<th>Leydig cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>67.9 ± 0.18</td>
<td>92.1 ± 0.18</td>
<td>100.6 ± 0.34</td>
<td>168.1 ± 0.58</td>
<td>44.4 ± 0.26</td>
<td>8.0 ± 0.21</td>
<td>9.3 ± 0.15</td>
<td>7.7 ± 0.15</td>
<td>6.3 ± 0.15</td>
<td>9.6 ± 0.16</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>67.3 ± 0.26</td>
<td>91.7 ± 0.20</td>
<td>99.9 ± 0.23</td>
<td>167.4 ± 0.42</td>
<td>43.8 ± 0.29</td>
<td>7.4 ± 0.16</td>
<td>9.0 ± 0.15</td>
<td>7.3 ± 0.15</td>
<td>6.0 ± 0.14</td>
<td>9.1 ± 0.10</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>66.5 ± 0.45</td>
<td>89.2 ± 0.44*</td>
<td>97.0 ± 0.29*</td>
<td>165.0 ± 0.42*</td>
<td>42.9 ± 0.18</td>
<td>6.6 ± 0.16</td>
<td>8.8 ± 0.13</td>
<td>6.8 ± 0.13</td>
<td>5.4 ± 0.16*</td>
<td>8.5 ± 0.22*</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>62.4 ± 0.26*</td>
<td>86.9 ± 0.23</td>
<td>94.3 ± 0.21*</td>
<td>163.2 ± 0.50*</td>
<td>39.2 ± 0.45*</td>
<td>6.3 ± 0.21</td>
<td>7.6 ± 0.16</td>
<td>6.7 ± 0.15</td>
<td>5.1 ± 0.10*</td>
<td>7.3 ± 0.15*</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>59.4 ± 0.16*</td>
<td>85.6 ± 0.16*</td>
<td>92.4 ± 0.34*</td>
<td>161.3 ± 0.15*</td>
<td>38.3 ± 0.30*</td>
<td>6.1 ± 0.18</td>
<td>7.1 ± 0.18</td>
<td>6.3 ± 0.22*</td>
<td>4.9 ± 0.10*</td>
<td>6.4 ± 0.16*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 5 animals

* Significant P ≤ 0.05 compared with control
Table 1.11  Temporal effects on biochemical contents of testis in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (days)</th>
<th>DNA</th>
<th>RNA</th>
<th>Protein</th>
<th>Glycogen</th>
<th>Cholesterol</th>
<th>Sialic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>2.51 ± 0.03</td>
<td>4.68 ± 0.02</td>
<td>147.7 ± 0.15</td>
<td>6.52 ± 0.02</td>
<td>9.25 ± 0.02</td>
<td>15.6 ± 0.16</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>2.48 ± 0.03</td>
<td>4.64 ± 0.02</td>
<td>147.3 ± 0.21</td>
<td>6.45 ± 0.03</td>
<td>9.33 ± 0.02</td>
<td>15.0 ± 0.21</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>2.42 ± 0.03</td>
<td>4.60 ± 0.03</td>
<td>146.8 ± 0.25*</td>
<td>6.41 ± 0.03*</td>
<td>9.45 ± 0.02*</td>
<td>14.3 ± 0.21*</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>2.29 ± 0.02*</td>
<td>4.13 ± 0.03*</td>
<td>141.2 ± 0.29*</td>
<td>6.19 ± 0.02*</td>
<td>9.65 ± 0.01*</td>
<td>13.3 ± 0.15*</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>2.11 ± 0.02*</td>
<td>3.87 ± 0.01*</td>
<td>135.5 ± 0.37*</td>
<td>5.53 ± 0.02*</td>
<td>9.81 ± 0.01*</td>
<td>12.5 ± 0.16*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 5 animals  
* Significant P < 0.05 compared with control

Table 1.12  Temporal effect on testis dehydrogenase and phosphatase enzymes activity in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (days)</th>
<th>LDH a</th>
<th>SDH b</th>
<th>3βHSD c</th>
<th>17βHSD d</th>
<th>Na+-K+ATPase e</th>
<th>Mg++ATPase e</th>
<th>Ca++ATPase e</th>
<th>ACP f</th>
<th>AKP f</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>9.06 ± 0.02</td>
<td>12.20 ± 0.20</td>
<td>0.44 ± 0.01</td>
<td>0.94 ± 0.01</td>
<td>7.45 ± 0.02</td>
<td>9.00 ± 0.04</td>
<td>6.10 ± 0.03</td>
<td>18.80 ± 0.30</td>
<td>14.10 ± 0.18</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>9.08 ± 0.02</td>
<td>12.12 ± 0.18</td>
<td>0.42 ± 0.02</td>
<td>0.93 ± 0.12</td>
<td>7.43 ± 0.01</td>
<td>8.94 ± 0.02</td>
<td>6.07 ± 0.01</td>
<td>18.70 ± 0.24</td>
<td>14.12 ± 0.10</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>9.10 ± 0.04</td>
<td>12.10 ± 0.22</td>
<td>0.37 ± 0.02*</td>
<td>0.83 ± 0.03*</td>
<td>7.32 ± 0.03*</td>
<td>8.90 ± 0.03</td>
<td>6.04 ± 0.01</td>
<td>18.65 ± 0.20</td>
<td>14.14 ± 0.12</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>10.14 ± 0.01*</td>
<td>11.40 ± 0.22*</td>
<td>0.35 ± 0.02*</td>
<td>0.75 ± 0.08*</td>
<td>7.10 ± 0.04*</td>
<td>7.75 ± 0.02*</td>
<td>5.80 ± 0.02*</td>
<td>17.70 ± 0.32*</td>
<td>14.90 ± 0.12*</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>10.30 ± 0.02*</td>
<td>11.15 ± 0.10*</td>
<td>0.32 ± 0.01*</td>
<td>0.72 ± 0.01*</td>
<td>6.42 ± 0.02*</td>
<td>7.10 ± 0.02*</td>
<td>5.42 ± 0.02*</td>
<td>16.20 ± 0.20*</td>
<td>15.50 ± 0.24*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 5 animals  
* Significant P < 0.05 compared with control

a  μmoles of pyruvate formed/ min/ g tissue  
b  μmoles of formazon formed/ min/ g tissue  
c  μmoles of NAD converted to NADH/ min/ g tissue  
d  μmoles of NADPH converted to NADP/ min/ g tissue  
e  μmoles of inorganic phosphorus formed/ min/ g tissue.  
f  μmoles of P-nitrophenyl formed/ min/ g tissue
Table 1.13 Temporal effect on oxidative stress parameters of testes in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (days)</th>
<th>Antioxidants</th>
<th>Oxidative stress byproducts</th>
<th>Oxidative stress enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GSH a</td>
<td>Ascorbic acid b</td>
<td>TBARS c</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>9.75 ± 0.06</td>
<td>362 ± 0.14</td>
<td>0.24 ± 0.14</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>9.73 ± 0.09</td>
<td>361 ± 0.13</td>
<td>0.26 ± 0.16</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>9.60 ± 0.06*</td>
<td>358 ± 0.05</td>
<td>0.32 ± 0.08*</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>9.56 ± 0.12*</td>
<td>332 ± 0.18*</td>
<td>0.37 ± 0.12*</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>8.72 ± 0.10*</td>
<td>308 ± 0.12*</td>
<td>0.44 ± 0.15*</td>
</tr>
</tbody>
</table>

a μmol of GSH/mg protein  
b μmol of ascorbic acid/gm wet tissue  
c nmoles MDA/mg of tissue protein  
d nmoles of protein carbonyl/mg protein  
e super oxide dismutase (SOD) unit/mg protein  
f μmol of H2O2/min/mg protein  
g μmole/min/mg protein  
* Significant P ≤ 0.05 compared to control.

Values are mean ± SEM of 10 animals.

Table 1.14 Temporal effects on biochemical contents of epididymis in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (days)</th>
<th>DNA</th>
<th>RNA</th>
<th>Protein</th>
<th>Glycogen</th>
<th>Cholesterol</th>
<th>Sialic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>2.40 ± 0.03</td>
<td>4.52 ± 0.02</td>
<td>220.2 ± 0.22</td>
<td>7.40 ± 0.16</td>
<td>8.40 ± 0.02</td>
<td>12.20 ± 0.42</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>2.38 ± 0.01</td>
<td>4.48 ± 0.02</td>
<td>215.6 ± 0.14</td>
<td>7.34 ± 0.08</td>
<td>8.44 ± 0.03</td>
<td>12.15 ± 0.22</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>2.36 ± 0.03</td>
<td>4.42 ± 0.03</td>
<td>211.2 ± 0.20</td>
<td>7.28 ± 0.12</td>
<td>8.50 ± 0.04</td>
<td>12.12 ± 0.24</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>2.30 ± 0.02*</td>
<td>4.08 ± 0.02*</td>
<td>199.2 ± 0.28*</td>
<td>6.68 ± 0.14*</td>
<td>9.60 ± 0.05*</td>
<td>11.40 ± 0.30*</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>2.10 ± 0.01*</td>
<td>4.00 ± 0.02*</td>
<td>190.1 ± 0.30*</td>
<td>6.60 ± 0.12*</td>
<td>9.90 ± 0.04*</td>
<td>11.12 ± 0.20*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 5 animals  
* Significant P ≤ 0.05 compared with control

100
Table 1.15 Temporal effects on epididymis dehydrogenase and phosphatase enzymes activity in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (days)</th>
<th>LDH</th>
<th>SDH</th>
<th>Na(^+)-K(^+) ATPase (^c)</th>
<th>Mg(^++) ATPase (^c)</th>
<th>Ca(^++) ATPase (^c)</th>
<th>ACP (^d)</th>
<th>AKP (^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>15.20 ± 0.18</td>
<td>14.12 ± 0.22</td>
<td>8.62 ± 0.15</td>
<td>10.70 ± 0.51</td>
<td>6.70 ± 0.10</td>
<td>15.40 ± 0.22</td>
<td>13.60 ± 0.40</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>15.14 ± 0.20</td>
<td>14.09 ± 0.26</td>
<td>8.58 ± 0.14</td>
<td>10.65 ± 0.40</td>
<td>6.62 ± 0.14</td>
<td>15.34 ± 0.18</td>
<td>13.62 ± 0.12</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>15.10 ± 0.26</td>
<td>13.95 ± 0.14</td>
<td>8.50 ± 0.20</td>
<td>10.60 ± 0.35</td>
<td>6.60 ± 0.32</td>
<td>15.29 ± 0.24</td>
<td>13.88 ± 0.28</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>16.00 ± 0.40*</td>
<td>13.20 ± 0.24*</td>
<td>7.50 ± 0.32*</td>
<td>9.96 ± 0.14*</td>
<td>6.16 ± 0.25*</td>
<td>14.60 ± 0.10*</td>
<td>14.70 ± 0.28*</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>16.70 ± 0.36*</td>
<td>12.84 ± 0.42*</td>
<td>7.10 ± 0.12*</td>
<td>9.51 ± 0.21*</td>
<td>5.96 ± 0.26*</td>
<td>14.32 ± 0.24*</td>
<td>15.36 ± 0.20*</td>
</tr>
</tbody>
</table>

* Significant P < 0.05 compared with control

<table>
<thead>
<tr>
<th>Enzyme activity (\mu)moles/ min/ g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>a (\mu)moles of pyruvate formed/ min/ g tissue</td>
</tr>
<tr>
<td>b (\mu)moles of formazone formed/ min/ g tissue</td>
</tr>
<tr>
<td>c (\mu)moles of NAD converted to NADH/ min/ g tissue</td>
</tr>
</tbody>
</table>

Table 1.16 Temporal effect on serum testosterone level in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (days)</th>
<th>Testosterone level (ng/ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>52.40 ± 0.45</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>51.38 ± 0.22</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>40.70 ± 0.40*</td>
</tr>
<tr>
<td>IV</td>
<td>20</td>
<td>31.80 ± 0.28*</td>
</tr>
<tr>
<td>V</td>
<td>30</td>
<td>20.62 ± 0.44*</td>
</tr>
</tbody>
</table>

Values are mean ± SE * Significant P ≤ 0.05 compared to control
Graph 1.1. Effect on testes and accessory reproductive organs weight in mice on exposure to methomyl

Graph 1.2. Effect on number of spermatogenic and Leydig cells in albino mice on exposure to methomyl
Graph 1.3. Effect on diameter of spermatogenic and leydig cells in albino mice on exposure to methomyl

Graph 1.4. Effect on biochemical contents of testis in albino mice on exposure to methomyl
Graph 1.5. Effect on testis dehydrogenase and phosphatase enzymes activity in albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Treatment (mg/kg/day)</th>
<th>LDH (µmoles/min/g tissue)</th>
<th>SDH</th>
<th>Na⁺-K⁺ ATPase</th>
<th>Mg⁺⁺ ATPase</th>
<th>Ca⁺⁺ ATPase</th>
<th>ACP</th>
<th>AKP</th>
<th>3βHSD</th>
<th>17βHSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>9</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>16</td>
<td>0.6</td>
<td>0.6</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>13</td>
<td>15</td>
<td>17</td>
<td>19</td>
<td>0.8</td>
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<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>16</td>
<td>18</td>
<td>20</td>
<td>22</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>21</td>
<td>22</td>
<td>23</td>
<td>24</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Significant
Graph. 1.6: Effect on testis oxidative stress parameters in albino mice on exposure to methomyl.
Graph 1.7. Effect on biochemical contents of epididymis in albino mice on exposure to methomyl

Graph 1.8. Effect on epididymis dehydrogenase and phosphatase enzymes activity in albino mice on exposure to methomyl
Graph 1.9. Effect on serum testosterone level in male albino mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Treatment (mg/kg/day)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td>■</td>
</tr>
</tbody>
</table>

Significant

Graph 1.10. Temporal effect on testes and accessory reproductive organs weight in mice on exposure to methomyl

<table>
<thead>
<tr>
<th>Treatment (days)</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td>■</td>
</tr>
</tbody>
</table>

Significant

mg/ml serum

Graph 1.9. Effect on serum testosterone level in male albino mice on exposure to methomyl

Graph 1.10. Temporal effect on testes and accessory reproductive organs weight in mice on exposure to methomyl
Graph 1.11. Temporal effect on number of spermatogenic and Leydig cells in albino mice on exposure to methomyl

Graph 1.12. Temporal Effect on diameter of spermatogenic and Leydig cells in albino mice on exposure to methomyl
Graph 1.13. Temporal effect on biochemical contents of testis in albino mice on exposure to methomyl

DNA RNA Glycogen Cholesterol Sialic acid Protein

Treatment (days)

Control 5 10 20 30 * Significant

(pg/ mg wet weight of tissue) (fimoles/ min/ g tissue)

Graph 1.14. Temporal effect on testis dehydrogenase and phosphatase enzymes activity in albino mice on exposure to methomyl

LDH SDH Na+-K+ ATPase Mg++ ATPase Ca++ ATPase ACP AKP

Treatment (days)

Control 5 10 20 30 * Significant

(μmoles/ min/ g tissue)
Graph. 1.15: Temporal effect on testis oxidative stress parameters in albino mice on exposure to methomyl

- GSH
  - μmole / mg protein
- Ascorbic acid
  - nmoles MDA/mg of tissue protein
- TBARS
  - nmoles of tissue protein carbonyl/mg
- Protein carbonyl
  - \( \text{μmole} / \text{min/mg protein} \)

Treatment (days)
- Control
- 5
- 10
- 20
- 30

* Significant
Graph 1.16. Temporal effect on biochemical contents of epididymis in albino mice on exposure to methomyl

![Graph 1.16](image)

**DNA**  **RNA**  **Glycogen**  **Cholesterol**  **Sialic acid**  **Protein**

<table>
<thead>
<tr>
<th>Treatment (days)</th>
<th>Control</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DNA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RNA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Glycogen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cholesterol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sialic acid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Significant

Graph 1.17. Temporal effect on epididymis dehydrogenase and phosphatase enzymes activity in albino mice on exposure to methomyl

![Graph 1.17](image)

**LDH**  **SDH**  **Na+ - K+ ATPase**  **Mg**  **Ca++ ATPase**  **ACP**  **AKP**

<table>
<thead>
<tr>
<th>Treatment (days)</th>
<th>Control</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
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<tbody>
<tr>
<td><strong>LDH</strong></td>
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<tr>
<td><strong>SDH</strong></td>
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<td><strong>Na+ - K+ ATPase</strong></td>
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<td><strong>Mg</strong></td>
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<tr>
<td><strong>Ca++ ATPase</strong></td>
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<td><strong>ACP</strong></td>
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<tr>
<td><strong>AKP</strong></td>
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</tbody>
</table>

* Significant

(pmoles/ min/ g tissue)

(fig/ mg wet weight of tissue)
Graph 1.18. Temporal effect on serum testosterone level in male albino mice on exposure to methomyl

Testosterone level

Treatment (days)

Control 5 10 20 30 * Significant

* Significant