CHAPTER - I

INDUCTION OF TESTIS TOXICITY, BIOCHEMICAL AND OXIDATIVE STRESS PARAMETER CHANGES IN ALBINO MICE AFTER EXPOSURE TO INDOXACARB
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INTRODUCTION

Pesticides have brought about the green revolution in the world and are being widely used to control agricultural pests and pests causing public health problems. Disorders of reproduction and hazards to reproductive health and associated functions have become prominent issues in recent decades after reports of adverse effects of certain chemicals on reproductive function. The male reproductive system is vulnerable to the effects of the chemicals and physical factors. This might be because sensitive events take place during spermatogenesis and the persistent environmental pollutants and/or physical factors may affect some of these events to some extent. Sometimes, people are exposed to various risk factors for reproduction such as toxic chemicals, radiation, intense heat, etc. in their workplace without knowing their exposure. Occupational exposure to these factors is generally higher than environmental exposure. However, in most cases, people are not aware of the hazard on reproduction due to these occupational exposures until they are interested in childbirth. The problem of infertility has increased from 8 to 15% over the past two decades in industrialized countries (Dondero et al., 1991; Runnebaum et al., 1997; Booker, 2000). A few studies are also available on the effects of multiple pesticides exposure on the reproductive system of male workers, which may affect the reproductive outcome. It has been reported among male workers who were exposed to various pesticides such as DDT, BHC, endosulfan; and organophosphorous pesticides i.e. malathion, methyl-parathion, dimethoate, monocrotophos, phosphamidon and quinalphos; synthetic pyrethroids such as fenvelrate and cypermethrin during mixing and spraying showed male mediated adverse reproductive defects (Rita et al., 1987; Rupa et al., 1991).

Global concerns have raised in recent years over the potential adverse effects that may result from exposure to chemicals that have the potential to interfere with the endocrine system which are called endocrine disrupting chemicals. The toxic insult to the testes can result in a multiplicity of effects, namely reduced sperm counts, concentrations and the production of defective spermatozoa, impaired androgen production and its consequences (Van Waeleghem et al., 1994; Irvine1994; Auger et al., 1995). Testis is the primary male sex organ with two well established function namely, spermatogenesis and steroidogenesis (Stainberger and Stainberger, 1978; Sharpe, 1987). When testes are exposed to environmental chemicals and drugs varying degrees of infertility may result (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.
There are reports that chlorinated pesticides endosulfan and dicofol adversely affects the male reproductive system in mice and rats (Hiremath and Kaliwal, 2000; Jadaramkunti and Kaliwal, 2002). There are other reports that mice exposed to trichloroethylene via inhalation (150 ppm) had increased percentages of abnormal sperm (Land et al., 1981; Van Waeleghem et al., 1994; Irvine1994; Auger et al., 1995). An increased percentage of abnormal sperm was observed in rats following drinking water exposure to trichloroethylene (9.5 ppm) in combination with other drinking water contaminations (Veeramachaneni et al., 2001). There are some reports that industrial chemicals tri-o-cresyl phosphate (TOCP) and the heavy metal cadmium resulted in various reproductive abnormalities such as testicular atrophy reduced epididymal sperm count and inhibition of several testicular enzymes in rats (Gunn et al., 1968; Sankuli et al., 1987) and it is also reported that tri-o-cresyl phosphate brings about testicular toxicity without any alterations in the circulating hormones, showing that the toxicity is due to its direct action on the reproductive structures (Sankuli et al., 1987). Among various phthalate esters, DEHP is one of the most widely studied toxicants in the male reproductive organs. Administration of DEHP reduces the fertility and induces testicular atrophy of laboratory animals (Oishi, 1979, 1986; Thomas, 1984). It was reported that the body weight was not altered by their methoxychlor (MXC) treatment, but testicular weight, epididymal weight, and number of spermatozoa in the tail of the epididymis were significantly reduced in a dose-dependent fashion (Chapin et al., 1997). Sharpe and Skakkeback (1997) have hypothesized that in utero exposure to environmental estrogens and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds may be responsible for decreased spermatozoa counts (concentration in human ejaculates) and other male reproductive tract disorders (Gray, 1995; Sharpe et al., 1998).

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). Sperm abnormalities and decreased sperm release in the epididymis along with a significant elevation of some testicular enzymes, was observed in animals treated with endosulfan (Sinha et al., 1996). Exposure to thiram caused time and dose
dependent morphological and biochemical changes in testes (Mishra et al., 1998). Serum cholesterol and testicular free sialic acid were increased and testicular protein decreased in the treated rats. Similar results also have been reported by intoxication of dicofol (Jadramkunti and Kaliwal, 2002), mancozeb (Ksheerasagar and Kaliwal, 2003) carbofuran (Ksheerasagar, 2005), and organophosphate pesticide phosphomidon in testes (Shreelakshmi, 2008).

It has been suggested that a number of factors exert their detrimental effects on testis through oxidative stress (OS). It has been shown that 1,1,1-trichloro o-2,2-bis (p-chlorophenyl) ethane (DDT) and related compounds share a mechanism of action similar to pyrethroids (Ellenhorn et al., 1997). Several studies have demonstrated that (DDT) and methoxychlor induce oxidative stress and lipid peroxidation (Gultekin, 2000; Gupta, 2001) and also the adverse effects on the male reproductive system, by decreasing the antioxidant enzymes in the epididymal sperm of goats (Gangadharan, 2001) and rats (Koner, 1998; Latchoumycandane, 2002; Kaur et al., 2006). Many studies have shown the oxidative effect of lindane in testis (Samanta, 1999).

Indoxacarb is a recently introduced oxadiazine insecticide derived from pyrazoline with activity against a wide range of pests (Harder et al., 1996). In insects, indoxacarb appears to be decarbomethoxylated to DCJW by an esterase/amidase (Wing et al., 2000). Several studies have demonstrated that DCJW is effective at blocking sodium channels at this target site (Zhao et al., 1999; Tsurubuchi et al., 2001; Narahashi, 2002; Tsurubuchi and Kono, 2003). However, indoxacarb and DCJW have also been shown to affect mammalian nicotinic acetylcholine receptors (Bachowski et al., 1998) and have a weak effect on mammalian gamma amino butyric acid (GABA) receptors (Tsurubuchi et al., 2001). Therefore, the present investigation was undertaken to study the effect of indoxacarb on testes and epididymides biochemical contents, enzymes activity and oxidative stress parameters in the testes in albino mice.
MATERIALS AND METHODS

Animals
Laboratory bred 3 to 4 months adult virgin female albino mice weighing about 20 to 30 gms showing regular estrous cycle of 4-5 days were used under standard animal housing condition (temperature controlled 25 ± 2°C facility and maintained with 12 hrs light/dark cycle) with unlimited access to pellet diet “Gold Mohar” (Hindustan Lever Ltd., Mumbai) and water ad libitum throughout the study in the animal house, P.G. Department of Studies in Zoology, Karnatak University, Dharwad. Animals were randomly divided into control and four treatment groups (Distilled water vehicle is served as control). Each group consists of 10 mice housed in separate polypropylene cages containing sterile paddy husk as bedding material. Daily phases of estrous cycle and body weights were recorded throughout the experiment.

Pesticide
The sample of indoxacarb (indoxacarb 14.5%) used in experiments was commercial insecticide supplied by E.I DuPont India Pvt., Ltd., Haryana obtained from the local company’s market containing Indoxacarb (a.i) 14.5 (w/w) in active enantiomer 6% (w/w) amorphous silicon dioxide 7% (w/w) polyethoxylated polyalyl phenol 9%(w/w) polyethoxylated polyalyl phenol phosphate 6%(w/w) distilled methyl soyate 57.5%(w/w). The graded doses of indoxacarb were prepared in distilled water vehicle to obtain desired concentrations. Indoxacarb was administered orally in distilled water vehicle, below their acute LD$_{50}$ level of intoxication in graded doses as 6, 12, 18 and 24 mg/kg body weight/ day dose 1/10$^\text{th}$ of LD$_{50}$ (Jacques and Bein, 1960) for 30 days to respective groups.

The graded dose of indoxacarb 6, 12, 18 and 24 mg/kg body weight/ day was administered orally for 30 consecutive days. The experiment was designed to determine the effective dose of indoxacarb on testes and epididymides, biochemical contents in testis and epididymis and oxidative stress parameters in the testis of albino mice.

All the experimental animals were autopsied by cervical dislocation on 31$^\text{st}$ day or 24 hrs after the terminal exposure. The testes and epididymides were dissected out 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 activity of enzymes SDH, LDH, 3β-
Histology and histometry

The testes were fixed in aqueous bovin’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). Randomly chosen 10 good sections from each testis in each group were observed under the microscope. 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 de Krester and Kerr (1994). The values were expressed as number and diameter of spermatogenic and Leydig cells per seminiferous tubules.

Biochemical estimations

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 0.1 N 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 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 trichloroacetic 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 hydroxylevulinylacedehyde. 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 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 as μmoles of formazon formed/ min/ g 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 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 C₁₉ and C₂₁ 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. 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

**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 1mmol 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 μmol sodium pyrophosphate buffer (pH 10.2),
40µl 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 filtrate was treated with an acid molybdate sodium which forms phosphomolybdic acid which was reduced by the addition of 1,2,1 amino naphthol 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 μmoles 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-nitrophenyl-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. 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 × 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 [17].

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.

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. $13.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$

**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 KH$_2$PO$_4$ 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 ($9.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$).

**Thiobarbaturic acid reactive substances (TBARS)**

The product of the reaction between malondialdehyde (MDA) and thiobarbutoric 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 $1.56 \times 10^5 \text{ cm}^{-1} \text{ M}^{-1}$ and was expressed in μmol TBARS/mg tissue protein. ($\varepsilon = 1.56 \times 10^2 \text{ mM}^{-1} \text{ cm}^{-1}$).

**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.
Protein carbonylation assay

This assay measures protein carbonyls, an indicator of protein oxidation, using 2, 4-dinitrophenylhydrazine (DNPH). 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

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).

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.

\[ X = \text{independent variable} \]
\[ n = \text{number of observations} \]
Whenever the numerical data provided, it is expressed as (X ± 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

a) Effect of indoxacarb on testes, spermatogenic and Leydig cells in mice

Testes weight (Table 1.1; Graph 1.1)

The mean weight of the testes in control mice was 830.8 mg. The mean weights of the testes with 6, 12, 18 and 24 mg indoxacarb treatments were 819.6, 799.4, 725.4 and 650.4 mg respectively. There was a significant decrease in testes weight with 18 and 24 mg indoxacarb treatments. However, 6 and 12 mg indoxacarb treatments showed no significant change in testes weight when compared with those of control mice.

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 70.47, 95.39, 103.51, 174.40 and 57.61 respectively and the mean diameter of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 8.59, 8.98, 7.58, 5.91 and 8.94 μm respectively.

In the mice treated with 6 mg indoxacarb, the mean numbers of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 65.26, 91.10, 96.10, 169.30 and 51.94 respectively and the mean diameter of spermatogonia, primary and secondary spermatocytes, spermatids and Leydig cells were 7.86, 8.15, 7.04, 5.21 and 8.68 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 control mice.

In the mice treated with 12 mg indoxacarb, the mean numbers of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 60.70, 87.30, 93.63, 164.40 and 45.94 respectively and the mean diameter of spermatogonia, primary and secondary spermatocytes, spermatids and Leydig cells were 6.80, 7.54, 6.55, 4.45 and 7.94 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 18 mg indoxacarb, the mean numbers of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 58.18, 85.20, 90.46, 160.02 and 38.51 respectively and the mean diameter of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 5.90, 6.75, 5.95, 3.61 and 7.13 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 24 mg indoxacarb the mean numbers of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 56.00, 83.10, 89.10, 158.01 and 30.18 respectively and the mean diameters of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and Leydig cells were 4.70, 5.83, 5.08, 2.88 and 6.87 μm respectively. There was 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 18 and 24 mg indoxacarb treatment. However, treatment with 6 and 12 mg indoxacarb 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.

**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 6 mg indoxacarb showed 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 12 mg indoxacarb exhibited decrease in the number of spermatogenic cells,
formation of giant cells, less number of sperms in the lumen of the seminiferous tubules. Leydig cells are in deformed conditions (Fig. 3). Histologic examination of the testis of the mouse treated with 18 mg indoxacarb showed formation of giant cells, significant decrease in the number of spermatogenic cells and lumen with loss of sperms. Leydig cells are in deformed condition (Fig. 4). Histologic observations of the testis of the mouse treated with 24 mg indoxacarb revealed formation of giant cells, vacuoles and marked reduction in spermatogenic cells. Lumen with tissue debris and loss of sperms, Leydig cells are in deformed condition (Fig. 5).

b) Effect on biochemical contents (DNA, RNA, protein, glycogen and cholesterol) of the testis in mice after exposure to indoxacarb (Table 1.3; Graph 1.4)

In the control mice the level of DNA, RNA, protein, glycogen and cholesterol was 2.30, 5.10, 160.01, 6.50 and 9.25 µg respectively in the testis. In the mice treated with 6 mg indoxacarb, the level of DNA, RNA, protein, glycogen, cholesterol was 2.19, 4.67, 156.35, 5.99 and 9.71 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 12 mg indoxacarb the level of DNA, RNA, protein, glycogen, cholesterol was 2.09, 4.35, 150.21, 4.80 and 10.46 µg respectively in the testis. There was no significant decrease in the levels of biochemical contents of the testis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 18 mg indoxacarb the level of DNA, RNA, protein, glycogen, cholesterol was 1.99, 3.58, 140.05, 4.20 and 11.01 µg respectively in the testis. There was significant decrease in the level of biochemical contents, except cholesterol where it was increased significantly in the testis when compared with that of the control mice.

In the mice treated with 24 mg indoxacarb the level of DNA, RNA, protein, glycogen, cholesterol was 1.85, 3.43, 130.04, 3.97 and 12.20 µg respectively in the testis. There was significant decrease in the level of biochemical contents, except cholesterol where it was increased significantly when compared with that of the control mice.

The findings of the present study on biochemical contents of the testis revealed that, in the mice treated with 18 and 24 mg indoxacarb caused significant decrease in the level of DNA,
RNA, protein and glycogen, whereas cholesterol content was increased significantly. In the mice treated with 6 and 12 mg indoxacarb, there was no significant change in the level of the biochemical contents of testis, when compared with those of the corresponding parameters of the control mice.

c) Effect on testis dehydrogenase (LDH and SDH) and phosphatase (ATPases, ACP and AKP) activity in mice after exposure to indoxacarb (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 8.50, 11.90, 0.37, 0.90, 7.70, 8.79, 6.20, 18.82 and 13.98 µmoles respectively in the testis. In the mice treated with 6 mg indoxacarb the activity of LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 8.68, 10.50, 0.35, 0.85, 7.48, 8.18, 5.98, 16.34 and 14.50 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 12 mg indoxacarb, the activity of LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 9.18, 9.20, 0.34, 0.79, 6.52, 7.26, 5.90, 15.98 and 15.01 µmoles respectively in the testis. There was no significant change in LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP when compared with those of the corresponding parameters of the control mice.

In the mice treated with 18 mg indoxacarb, the activity of LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 9.97, 8.15, 0.30, 0.73, 5.48, 6.30, 5.70, 15.25 and 15.35 µmoles respectively in the testis. There was 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 24 mg indoxacarb, the activity of LDH, SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 10.31, 7.07, 0.28, 0.65, 4.58, 6.20, 5.35, 15.07 and 15.74 µmoles respectively in the testis. There was significant decrease in the activity of SDH, 3βHSD, 17βHSD, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺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 18 and 24 mg indoxacarb 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 mice treated with 6 and 12 mg indoxacarb caused no significant change in the activity of enzymes, compared with those of the corresponding parameters of the control mice.

d) Antioxidants and oxidative stress byproducts of the testis in mice after exposure to indoxacarb (Table 1.5; Graph 1.6)

In the control mice the level of GSH, TBARS, protein carbonyl and ascorbic acid was 1.75, 0.65, 1.10, and 300 μg respectively in the testis. In the mice treated with 6 mg indoxacarb, the level of GSH, TBARS, protein carbonyl and ascorbic acid was 1.70, 0.75, 1.18, and 280 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 12 mg indoxacarb the level of GSH, TBARS, protein carbonyl and ascorbic acid was 1.55, 0.95, 1.28 and 250 μg respectively in the testis. There was a no significant decrease in the levels of antioxidants and oxidative stress byproducts contents when compared with those of the corresponding parameters of the control mice.

In the mice treated with 18 mg indoxacarb the level of GSH, TBARS, protein carbonyl and ascorbic acid was 1.43, 1.28, 1.40 and 200 μg 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 in the testis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 24 mg indoxacarb the level of GSH, TBARS, protein carbonyl and ascorbic acid was 1.30, 1.55, 1.65, and 172.00 μg 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.

The findings of the present study on biochemical contents of the testis revealed that, in the mice treated with 18 and 24 mg indoxacarb 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 6 and 12 mg indoxacarb, there was no significant change in the levels of the antioxidants and oxidative stress byproducts of testis when compared with those of the corresponding parameters of the control mice.

e) Oxidative stress enzymes (catalase (CAT), super oxide dismutase (SOD) and glutathione s-transferase GST) of the testis in mice after exposure to indoxacarb (Table 1.5; Graph 1.7)

In the control mice, the activity of catalase, SOD, and GST was 0.020, 58.05, and 0.90 μmoles respectively in the testis. In the mice treated with 6 mg indoxacarb the activity of catalase, SOD, and GST was 0.022, 61.15 and 0.93 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 12 mg indoxacarb, the activity of catalase, SOD, and GST was 0.025, 66.16 and 0.96 μmoles respectively in the testis. There was no significant change in catalase, SOD, and GST when compared with those of the corresponding parameters of the control mice.

In the mice treated with 18 mg indoxacarb, the activity of catalase, SOD, and GST was 0.029, 68.20 and 0.98 μmoles respectively in the testis. There was significant increase in the activity of catalase, SOD, and GST, when compared with those of the corresponding parameters of the control mice.

In the mice treated with 24 mg indoxacarb, the activity of catalase, SOD, and GST was 0.033, 73.20 and 1.02 μmoles respectively in the testis. There was significant increase in the activity of catalase, SOD, 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 18 and 24 mg indoxacarb showed significant increase in the activity of catalase, SOD, and GST. In the mice treated with 6 and 12 mg indoxacarb caused no significant change in the activity of enzymes, when compared with those of the corresponding parameters of the control mice.

f) Effect of indoxacarb on epididymides weight in mice (Table 1.1; Graph 1.1)

The mean weight of the epididymides in control mice was 390.0 mg. The mean weights of the epididymides with 6, 12, 18 and 24 mg indoxacarb treatments were 345.4, 290.3, 279.0 and 240.4 mg respectively. There was a significant decrease in epididymides weight with 18 and 24 mg indoxacarb treatments. However, treatment with 6 and 12 mg indoxacarb caused no significant change in epididymides weight when compared with those of control mice.

g) Effect on biochemical contents (DNA, RNA, protein, glycogen and cholesterol) of the epididymis in mice after exposure to indoxacarb (Table 1.6; Graph 1.8)

In the control mice the level of DNA, RNA, protein, glycogen and cholesterol was 3.10, 5.90, 210.29, 7.50 and 7.78 µg respectively in the epididymis. In the mice treated with 6 mg indoxacarb, the level of DNA, RNA protein, glycogen and cholesterol was 2.95, 5.55, 205.59, 7.20 and 8.50 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 12 mg indoxacarb the level of DNA, RNA, protein, glycogen and cholesterol was 2.50, 4.55, 195.40, 6.92 and 8.98 µg respectively in the epididymis. There was no significant change in the level of biochemical contents, cholesterol content where it was increased in the epididymis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 18 mg indoxacarb the level of DNA, RNA, protein, glycogen and cholesterol was 2.20, 4.40, 180.69, 6.73 and 9.52 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 24 mg indoxacarb the level of DNA, RNA, protein, glycogen and cholesterol was 1.90, 4.20, 169.83, 6.45 and 10.35 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 18 and 24 mg indoxacarb caused significant decrease in the level of DNA, RNA, protein, and glycogen and, whereas cholesterol level was increased significantly. Treatment with 6 and 12 mg indoxacarb showed no significant change in the level of biochemical contents, when compared with those of the corresponding parameters of the control mice.

h) Effect on epididymis dehydrogenase (LDH and SDH) and phosphatase (ATPases, ACP and AKP) activity in mice after exposure to indoxacarb (Table 1.7; Graph 1.9)

In the control mice the activity of LDH, SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 13.45, 12.50, 8.70, 10.36, 6.98, 13.78 and 14.08 μmoles respectively in epididymis. In the mice treated with 6 mg indoxacarb the activity of LDH, SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 13.98, 11.78, 8.07, 9.87, 6.56, 12.88 and 14.76 μmoles respectively in the epididymis. There was no significant change in the activity of enzymes in epididymis when compared with those of the corresponding parameters of the control mice.

In the mice treated with 12 mg indoxacarb the activity of LDH, SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 14.93, 10.93, 7.29, 9.12, 5.97, 12.08 and 15.33 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 18 mg indoxacarb the activity of LDH, SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP and AKP was 16.09, 10.03, 6.39, 8.26, 5.37, 11.31 and 16.00 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 24 mg indoxacarb the activity of LDH, SDH, Na⁺-K⁺ATPase, Mg²⁺ATPase, Ca²⁺ATPase, ACP and AKP was 17.27, 8.98, 5.23, 7.14, 4.00, 10.38 and 16.94 μmoles respectively in the epididymis. There was significant decrease in the activity of SDH, Na⁺-K⁺ATPase, Mg²⁺ATPase, Ca²⁺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 18 and 24 mg indoxacarb, caused significant decrease in the activity of SDH, Na⁺-K⁺ATPase, Mg²⁺ATPase, Ca²⁺ATPase and ACP in the epididymis, whereas the activity of LDH and AKP were increased significantly. The mice treated with 6 and 12 mg indoxacarb caused no significant change in the enzymes activity of epididymis when compared with those of the corresponding parameters of the control mice.

i) Agarose gel electrophoresis of cellular DNA in the testis of mice after exposure to indoxacarb

The DNA gel electrophoresis analysis in testes of the control and with 12, 18 and 24 mg/kg body weight indoxacarb treated mice is shown in Lane 1, 2, 3 and 4 respectively (Fig. 1). The results reveled that the DNA band was observed in lane 1 of the cells in the testes of control mice. However, lane 2, 3 and 4 showed smear of DNA in the tissue of the testes in mice treated with 12, 18 and 24 mg/kg body weight of indoxacarb for 30 days respectively indicates necrosis of the cells.
DISCUSSION

a) Effect of indoxacarb on testes and epididymides in mice

The testes 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). Spermatogenesis and steroidogenesis are the major functions of testis controlled (Steinberger and steinberger, 1975; Sharpe, 1987). Testis is a complex organ containing germ cells, Sertoli cells and Leydig cells and leutenizing hormone (LH) stimulates Leydig cells to produce testosterone (Neaves, 1977; Ewing and Zirkin, 1983). 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 leutenizing 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.

Genital organ weights were the criteria used to evaluate the reproductive toxicity of the tested pesticides to rats and mice. Perusal of the present results clearly exhibited that the effect of indoxacarb pesticide on testes and epididymis weights was dose dependant since the tested pesticide at 18 and 24 mg/kg/body wt significantly decreased these weights. However, with 6 and 12 mg of indoxacarb treatment caused no significant change in the weight of testes and epididymis. Similar results have been reported by intoxication of dicofol (Jadramkunti and Kaliwal, 2002), mancozeb (Ksheerasagar and Kaliwal, 2003), carbosulfan (Ksheerasagar, 2005), and organophosphate pesticide phosphomidon (Shreealakshmi, 2008).

Useful information on male reproductive capacity of laboratory animals can be obtained by measuring weights and volume of testes, prostate, seminal vesicles, epididymis and coagulating glands (Doul et al., 1980). The weights of testes and accessory sex organs are known to be reliable indices of testicular androgen production (Price and Williams-Ashman, 1961; Rind et al., 1963). Sublethal chronic administration of quinolophos resulted in decreased
testicular mass (Sarkar, 2000). Significant decline in testicular weight may due to decrease in the number of spermatogenic elements and spermatozoa (Sherins and Hawards, 1978; Takihara et al., 1987). Ray (1991) has indicated that relative weights of accessory reproductive glands depend on testosterone level (Jana et al., 2003), Several pesticides have reduced the organ weights by affecting either hypothalamus or pituitary or both (Okazaki et al., 2001; Lacthoumycandane et al., 2002).

The testes of control mice exhibited normal testicular structure on histological examination. However, high concentration of indoxacarb showed formation of giant cells, vacuoles, decreased number of spermatogenic cells, lumen with tissue debris and loss of sperms, Leydig cells are in deformed conditions. Similar results have been reported in mice and rats treated with pesticides. In contrast to our above finding it has been also reported that histopathologically the testes in all dichlorovos (DDVP) treated groups were not significantly different from control groups (Okamura et al., 2005). No histological changes appeared in mice testes with 7/mg/kg/day dimethoate treated groups with compared to the control group (Farag et al., 2007). It has been shown that profenophos caused congestion in testes blood vessels with edema among seminiferous tubules in male rats with high dose. Further it has been also reported that histopathological examination revealed atrophied seminiferous tubules and degenerative changes of sertoli cells at 0.4 mg/kg/day carbofuran treated rabbits (Pant et al., 1997). Carter et al., (1984) have revealed that benomyl in the pubertal and post pubertal rats resulted in decreased testicular weight and sperms in epididymis and vas deferens. The carbamate fungicides, manebo 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).

The epididymis is the organ within which sperm undergo the final stages of maturation, gaining motility and the ability to fertilize oocytes in vivo (Amann et al., 1993). Along with the processes of sperm maturation, the epididymis may play a role in protecting sperm during epididymal transit (Hinton et al., 1995). There are reports that the epididymis contains glutathione s-transferases (GSTs), cytochrome P450 2E1 (CYP2E1), and alcohol dehydrogenase (DuTeaux et al., 2003; Tietjen et al., 1994; Veri et al., 1994). The presence of these enzymes supports the notion that the epididymis may play a role in the detoxification of xenobiotic compounds in situ. Alternatively, these enzymes can bioactivate certain chemicals, producing
reactive and potentially more toxic intermediates from the parent compound (Yost, 2001). The relative amount and activity of bioactivating versus detoxifying enzymes can greatly influence the likelihood of toxicity in a target organ (Yost, 2001). Physiological and biochemical integrity of epididymal canal is dependent on androgens (Setty et al., 1977; Brooks, 1974). It has been 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).

Therefore, in the present study with increasing dose of indoxacarb the decrease in testes and epididymides weight may be due to

a) The indoxacarb may act like an antigonadal action 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; Latchoumycandane et al., 2002).

b) Indoxacarb may affect the 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).

c) It may have a 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 organophosphate insecticide quinolphos (Sarkar et al., 2000). It has been revealed that pesticides also induce inhibition of the acetyl cholinesterase (AchE) which in turn might increase the concentrations 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 gonadal steroids which are essential for normal functioning of the gonads (Sharpe, 1987)

d) Indoxacarb may affect the level of testosterone as it has been suggested that 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).

e) It may inhibit the release of GnRH as it has been reported 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 nor epinephrine and the nor epinephrine then stimulates the release of GnRH. Thus GnRH release is affected through the inhibition of DβH (Maj and Vetulani, 1969; Prezewlocka et al., 1975).

b) Effect of indoxacarb on number and diameter of spermatogenic (spermatogonia, spermatocytes, spermatids) and Leydig cells in mice

The present graded dose exposure study on number and diameter of spermatogenic and Leydig cells revealed that the treatment with 18 and 24 mg indoxacarb caused significant decrease in the number and diameter of spermatogenic and Leydig cells. However, treatment with 6 and 12 mg of indoxacarb caused no significant change in number and diameter of spermatogenic and Leydig cells. Similar results have been reported by intoxication of dicofol (Jadramkunti and Kaliwal, 2002), mancozeb (Ksheerasagar and Kaliwal, 2003), earbosulfan (Ksheerasagar, 2005), and organophosphate pesticide phosphomidon (Shreelakshmi, 2008). Similar results were reported on treatment with carbaryl a carbamate insecticide known to cause sperm abnormalities, reduction in number of spermatogonia, spermatozoa and degeneration of Leydig cells in rats and mice (Degrave et al., 1976; Kitagava et al., 1977; Srivastava and Srivastava, 1998). It has been reported that carbofuran a carbamate pesticide causes dose dependent reduction number of epididymal sperm count and sperm motility with increasing abnormal sperms numbers (Pant et al., 1995). It has been revealed that in 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 pyknotic nuclei and reduced nuclear size (Shivanandappa and Krishnakumari, 1983). 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 and secondly by chromosomal damage due to inhibition of DNA synthesis leading to formation of giant cells.

It has been suggested that apoptosis is a physiological process of cell death leading to the controlled elimination of single unwanted cell from middest viable tissue without damaging the neighboring unaffected cells (Pesel et al., 1993; Averal et al., 1996). Carter et al., (1987) have reported the spermatogenic inhibition in rats treated with carbamate fungicide carbendazin 4 mg/kg/day for 10 days. Histologic examination of testis 45 days post exposure revealed severe seminiferous tubular atrophy. 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. Similar results were also have been reported in decreased testicular weight decreased in number and diameter of spermatogenic and Leydig cells in rats and mice treated with pesticides disulfiran and its metabolite dithiocarbamate (Maj and Vetulani, 1969; Prezewlocka et al., 1975).

In the present study, the histologic observations of the testis of the mouse treated with 18 and 24 mg indoxacarb reveals formation of giant cells decreased number of spermatocytes, spermatids and sperms in the lumen. Leydig cells are in deformed condition. However mouse treated with lower dose of indoxacarb no significant change in the number and diameter of spermatogenic cells interstitial tissue contains clusters of Leydig cells. Similar results have been reported to cause decreased number spermatogenic cells, lumen with loss of sperms and Leydig cells are in deformed condition with treatment of carbaryl (Degrave et al., 1976; Kitgawa et al., 1977; Shrivastava and Shrivastava, 1998) carbofuran (Pant et al., 1995) and pentylether, 1,4-diethoxybutane and 1,6-dimethoxyhexane (Poon et al., 2004)

In conclusion, the observed results indicates that high dose and prolong exposure of indoxacarb affect the spermatogenesis showing antispermatogenic and anti-androgenic property as reflected by the effect on number and diameter of spermatogenic and Leydig cells, testes weights as testicular steroids influences spermatogenesis and function of accessory reproductive organs (Stroker et al., 2000; Okazaki et al., 2001; Latchoumycandane 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; Maitra and Sarkar et al., 1996; Sarkar et al., 2000). Maitra and Sarkar (1996) and Sarkar et al., (2000) have observed decreased acetylcholinesterase activity along with severe testicular damage in methyl parathion munias and quinolphos treated rats. Thus it is presumable that the testicular damage induced in the present study might have relation with decreased acetylcholinesterase activity. It is unknown at present whether or not any effects of indoxacarb on the nerve supplying these reproductive organs mediates the structural changes observed in the present study. However, further investigation is needed in this regard.

c) Effect on biochemical contents (DNA, RNA, protein, glycogen and cholesterol) of the testis and epididymis in mice after exposure to indoxacarb

Nucleic acids

Nucleic acids are of two types RNA and DNA these are most important macromolecules carry all kinds of necessary biological information and are involved in gene action, which is essential in regulation of cell metabolism and expression of the characters within synthesized in nucleus but mainly found in cytoplasm to carry out the protein synthesis. RNA plays vital role in cell metabolism by producing many proteins to catalyze the various biochemical reactions.

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 18 and 24 mg indoxacarb. However, treatment with lower doses of indoxacarb showed no significant change in the levels of nucleic acids in the testis and epididymis. Similar results have been observed in rats exposed to lindane (Sujatha et al., 2001). Similar results have been reported by intoxication of dicofol (Jadramkunti and Kaliwal, 2002), mancozeb (Ksheerasagar and Kaliwal, 2003) carbosulfan (Ksheerasagar, 2005), and organophosphate pesticide pesticide phosphomidon (Shreelakshmi, 2008).

It has been revealed that organophosphorous pesticides such as chloropyrifos, acephate, monocrotophos, methyl parathion and carbamate pesticides such as captan, furdan, carbosulfan,
aldicarb, benomyl, carbofuran and nitrosopropoxur causes 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; Saxena et al., 1997; Rupa et al., 1991; Rahman et al., 2002; Topktas et al., 1996). The main toxic effect of organophosphorous 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). 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. Plasmatic membranes of spermatozoa contain high concentrations of polyunsaturated fatty acids and therefore, are highly sensitive to oxidative stress. The oxidative stress in testicular tissue and measured the level of malondialdehyde (MDA), it has been shown that several pesticides causes (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 are implicated as possible causes of increased infertility in males (Pina-Guzman et al., 2006). 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). Meiosis is a process that eventually results in chromosome reduction from diploid to haploid state. This lengthy phase between primary to secondary spermatocytes production is essential for genetic continuity; thus, this period is thought to be highly susceptible to environmental insults and can result in chromosomal lesions (Adler, 1982). Marchetti et al., (2001) have found structural aberrations and uneuploidy in spermatocytes from male mice treated with an agent used in cancer thermotherapy, effects that were transmitted to the zygotes. Similarly RNA synthesis known to alter 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 of enzymes, RNA synthesis would inturn influences the level of protein synthesis. Thus the total RNA content of an organ is an index of functional status (Coffey et al., 1968).

In the present study, the observed results in the levels of nucleic acids in the testis and epididymis under the influence of indoxacarb treatment in mice may be due to
a) Decrease in mitotic index and disturbed cell division (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 (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) Due to its 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 (Lyons, 1999; Sarkar et al., 2001; Nagvi et al., 1992).

d) Due to inhibition of DBH (Dopamine-β-hydroxylase) and release of GnRH 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 dehydrotestosterone (Coffey et al., 1968). Therefore, the decreased DNA and RNA in the testis and epididymis may be due to genotoxic action (or) oxidative stress of indoxacarb or decreased levels of testosterone through its effect on central nervous system and gonads.

Proteins and Glycogen

Proteins are molecules that have different variety of functions in different ways in the cell as enzymes, structural proteins, antibodies, transport molecules and many other functions. Enzymes catalyze all cellular reactions, which make them extremely important in cell activity. Liver is the major source for synthesis of serum proteins (with the exception of immunoglobulin). A decrease of serum protein results from protein loss during hemorrhage and starvation. In sufficient 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 may be due to the intestinal or pancreatic dysfunction resulting in mal-absorption, which leads to malnutrition (Kaneko et al., 1966).

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 tissues (Stetten, 1956;
Mayer, 1977). On the other hand liver plays an important role in glucose metabolism and it is major site of insulin clearance (Duckworth et al., 1988; Michael et al., 2000).

In the present graded dose exposure study, the mice treated with 18 and 24 mg indoxacarb treatment showed significant decrease in the levels of protein and glycogen in the testis and epididymis. However, the mice treated with lower doses of indoxacarb showed no significant change in testis and epididymis. Similar observations have been made in rats and mice exposed to monocrotophos, methyl parathion, hexachlorohexane, carbaryl and mancozeb (Desta, 1994; Ratnasooriya et al., 1996; Reena Kacker et al., 1997; Sanjay et al., 1998; Prashanti et al., 2006; Ferdinand et al., 2007). Similar results have been reported by intoxication of pesticides (Jadramkunti and Kaliwal, 2002; Ksheerasagar and Kaliwal, 2003; Ksheerasagar, 2005; Shreelakshmi, 2008).

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. It has been reported that due to intoxication of Pirimiphos-methyl is an organophosphate pesticide there is significant decrease in the protein level (Ferdinand et al., 2007). Protein levels decreased treatment of 3.5 mg/kg, indicating that the oral exposure is effective in reducing protein synthesis in the epididymis (Prashanthi et al., 2006) due to intoxication of methyl parathion. Significant decrease in protein level might be due to catabolism of proteins and/ or malfunctioning of liver (Harper et al., 1977) or which results in the inhibitory effect on the activities of enzymes involved in the androgen biotransformation (Dikshith and Datta, 1972). The principal cells of the epididymal epithelium synthesize proteins which are androgen dependent (Killian et al., 1973).

It has been reported that mancozeb changes the biochemical parameters of the reproductive tract. A fall in glycogen level may be due to interference in glucose metabolism. Fungicides induce inhibition of glycolytic enzymes, which may affect the maturational process of spermatozoa and their motility. Inhibition of glycogen synthesis eventually decreases spermatogenesis process (Desta, 1994). 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-
Meyer-hoff 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 study, it has been found that with increasing dose exposure to indoxacarb
caused decrease in the levels of protein and glycogen. In the testes and epididymis may be due to

a) Genotoxic action of indoxacarb 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)
b) Lower content of protein and glycogen content was possibly due to direct effect of
indoxacarb on protein and glycogen metabolism or due to enhanced proteolytic activity as
a consequence of increased metabolic demands following exposure to the toxic stress
c) 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 synthesis takes place in liver. A small fraction of the cholesterol
is incorporated in to the membranes of hepatocytes, but most of it is exported in one of two
forms; bile acids or cholesteryl esters. It 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 is 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. Cholesterol can be
cleaved into pregnenolone by cytochrome P450 (SCC) (side chain cleavage). Further successive
steps continued for steroidogenesis. 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.

Most of the Cholesterol is derived from lipoproteins or from high density proteins. Free cholesterol is insoluble in cytosol and is transported into mitochondria by sterol carrier protein. Once it is reached the mitochondria cholesterol can be cleaved into pregnenolone by cytochrome P450 (side chain cleavage). Cholesterol being precursor for steroid hormones and also for vitamin D which is essential for regulation of calcium and phosphorus metabolism and bone growth, cholesterol essential for membrane synthesis. Most of the cholesterol synthesis takes place in liver. A small fraction of the cholesterol made there is incorporated into the membranes of hepatocytes, but most of it is exported in one of two forms; bile acids or cholesteryl esters. Bile acids and their salts are relatively hydrophilic cholesterol derivatives that are synthesized in the liver and aid in lipid digestion. The amount of fat present in any tissue is the net effect of deposition and mobilization. The fat that is deposited in various tissues may be derived from either a 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. Cholesterol levels are considered valuable indicators of drug induced disruption of lipid metabolism and development of fatty liver and altered cholesterol levels are implicated in impaired biliary excretion. An increase in cholesterol level is the sign of liver damage (Thibodeau et al., 1999). Several studies have revealed that chemically induced toxicity potentially alters liver biochemical contents.

In the present graded dose exposure study, the mice treated with 18 and 24 mg indoxacarb showed significant increase in the level of cholesterol in testis and epididymis. However, treatment with 6 and 12 mg indoxacarb caused no significant change in the cholesterol level. Similar results have been observed in rats treated with monocrotophos, cythion and tamaron (Adilaxamamma et al., 1995; Hanaby et al., 1991) Similar results have been reported by intoxication of pesticides (Jadramkunti and Kaliwal, 2002; Ksheerasagar and Kaliwal, 2003; Ksheerasagar, 2005; Shreelakshmi, 2008).

Treatment of Pirimiphos-methyl (PM) rats for 90 days with various doses of PM, the total cholesterol level was decreased in the serum and increased in the testis. It is well known that cholesterol is the main precursor for steroid genesis and it is produced mostly in the liver.
from LDL and HDL (Robel, 2001; Johnson, 2002). The increase in testicular concentration of total cholesterol level is almost an index of reduced testosterone, the main hormone for control of fertility of animals. Dimethoate blocks the steroid hormone biosynthesis in the Leydig cells (Thonneau et al., 1996). The increase in testicular concentration of total cholesterol level is almost an index of reduced steroidogenesis (Dorfman et al., 1963). Treatment with Mancozeb also changes increased concentration of cholesterol in testes suggests that impairment of spermatogenesis is due to decreased androgen concentration (Bedwal et al., 1994). 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., 1963).

The results of the present study indicated that high doses of indoxacarb treatment caused increase in cholesterol level of the testis and epididymis which may be due to

a) Inhibition of steroidogenesis in testis and adrenal (Shivanandappa and Sarkar, 1979; Shivanandappa and Krishnakumari, 1981; Chitra et al., 1987; Dorfman et al. 1997; Sujatha et al., 2001).

b) 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, the reduced activity of the important enzyme 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.

d) Effect of indoxacarb on lactate dehydrogenase (LDH) and succinic dehydrogenase (SDH) activity of testis and epididymis in mice

LDH is commonly used marker enzyme for lethal cell injury (Lock et al., 1993; Valentovic and Ball, 1998; Lash et al., 1995). Most of the cells contain LDH and when these cells are lethally injured, loss of membrane integrity can be assed 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). The increased LDH activity leads to accumulation of pyruvate. Pyruvate then enters the Krebs cycle and gets reduced to CO$_2$ and water yielding ATP molecules in aerobic condition since it needs oxygen for oxidation. Thus the formation of pyruvate was favored over lactate. This may indicate glycolysis under aerobic in erythrocytes and skeletal muscles pyruvate is converted to lactate. This may indicate glycolysis under aerobic conditions in the tissue. During anaerobic glycolysis in erythrocytes and skeletal muscles pyruvate is converted to lactate at rate of oxidation of NADH to NAD$^+$ and the reaction is catalyzed by enzyme lactate dehydrogenase. 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. An increase in cerebrospinal fluid LDH activity has been reported in the presence of tumors 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). LDH is involved in glucose metabolism and is widely distributed in all organ cells of the body. 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. Lactate dehydrogenase (LDH) is involved in glucose metabolism and is widely distributed in all organ cells of the body especially in cardiac, skeletal muscle, liver, kidney and red blood cells. The elevated LDH activity is a marker enzyme for lethal cell injury (Lock et al., 1993; Valentovic and Ball, 1998; Lash et al., 1995) and tissue damage and serves as a good diagnostic tool in toxicology. The increased LDH activity leads to accumulation of pyruvate. Pyruvate then enters the Krebs cycle and gets reduced to CO$_2$ and water yielding ATP molecules in aerobic condition since it needs oxygen for oxidation. Thus the formation of pyruvate was favored over lactate. This may indicate glycolysis under aerobic in erythrocytes and skeletal muscles pyruvate is converted to lactate. This may indicate glycolysis under aerobic conditions in the tissue. During anaerobic glycolysis in erythrocytes and skeletal muscles pyruvate is converted to lactate at rate of oxidation of NADH to NAD$^+$ and the reaction is catalyzed by enzyme lactate dehydrogenase (LDH).
Succinic dehydrogenase enzyme associated with tissue having high metabolic activity engaging in absorptive or secretory activity (Padykula, 1952). This enzyme bound to inner surface of the inner mitochondrial membrane. This enzyme is an important enzyme in citric acid cycle and it catalyses the oxidation of Succinate to fumarate. The fumarate formed further converted into malate and then oxalate, thus completes one TCA cycle. This enzyme 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). Blackshaw and Samisoni (1967) have reported that bull and guinea pig testes the spermatozoa were more active than the interstitial tissue. SDH is an important enzyme in citric acid cycle and it catalyses the oxidation of succinate to fumarate. The change in SDH activity reflects the effect on TCA cycle. This enzyme bound to inner surface of the inner mitochondrial membrane. This mitochondrial enzyme is associated with late stages of spermatogenesis. Succinic dehydrogenase is an enzyme associated with tissue having high metabolic activity engaging in absorptive or secretory activity (Padykula, 1952).

In the present graded dose 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 18 and 24 mg indoxacarb. However, with 6 mg and 12 mg indoxacarb treatment caused no significant change in the activity of LDH and SDH in the testis and epididymis. Similar results have been reported by intoxication of carbofuran (Ksheerasagar, 2005), methomyl and organophosphate pesticide phosphomidon (Sudheer, 2006; Shreelakshmi, 2008). Similar findings have been reported in rats and mice treated with different pesticides (Kacker et al., 1997; Mishra et al., 1998; Mehgoub and El-Medney, 2001; Altuntas et al., 2002; Pant and Srivastava, 2003; Kuladip Jana et al., 2006).

Similarly carbosulfan showed increase activity of LDH, decreased activity of SDH in mice. Increased permeability of cell and necrosis are usually characterized by rise in LDH activity (Radhiah, 1985). Similar reports are described in different animal species in response to heavy metals and pesticides (Natarajan et al., 1984; Sastry et al., 1988; Sastry et al., 1994; Altuntas et al., 2002). Moreover, several investigators have reported that the oxygen
consumption and the activities of testes respiratory Succinic dehydrogenase (SDH), malate dehydrogenase (MDH), and Nicotine adenine iso-decarboxylase (NAD-Iso De), were decreased with the elevation of glucose-6-phosphate dehydrogenase, glyceraldehyde dehydrogenase and/or LDH activities in stressed animals. They suggested that the stressed animals are meeting its energy requirements through anaerobic oxidation (Balavenkatasubbaiah et al., 1984; Prasada et al., 1985; Bhaskaran et al., 1988; Rajeswari et al., 1989; Gerbracht et al., 1990; James et al., 1996; Sharma et al., 1990; Reddy et al., 1994; Vaglio et al., 1999;) additionally (Sharma. and Gupta, 1990) reported decrease in the activity of testes SDH suggests that anaerobic metabolism was favored over aerobic oxidation of glucose through Krebs cycle in order to mitigate the energy crisis for survival.

In the present study, it has been found that with increase in dose exposure to indoxacarb caused significant increase in the activity of LDH and decrease in the SDH activity in the testis and epididymis. 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; Freidkalns and Weber, 1968) as this enzyme is related with high metabolic activity such as absorption and secretion (Padykula, 1952).

b) 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). They suggested that the stressed animals are meeting its energy requirements through anaerobic oxidation (Balavenkatasubbaiah et al., 1984; Prasada et al., 1985; Bhaskaran et al., 1988; Rajeswari et al., 1989; Gerbracht et al., 1990; James et al., 1996; Sharma et al., 1990; Reddy et al., 1994; Vaglio et al., 1999;)

c) Decrease in the testosterone level have affected the activity of SDH (Srivastava and Vijayan, 1996)

e) Effect of indoxacarb on 3-β-hydroxysteroid dehydrogenase (3-βHSD) and 17-β-hydroxysteroid dehydrogenase (17-βHSD) activity of the testis in mice

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).

The results of the present study revealed that the treatment of graded dose exposure of indoxacarb with 18 and 24 mg showed significant decrease in 3β-HSD and 17β-HSD activities in the testis. However, treatment with 6 and 12 mg indoxacarb showed no significant change in 3β-HSD and 17β-HSD activities in the testis. Similar results have been reported by intoxication of carbofuran (Ksheerasagar, 2005), and organophosphate pesticide phosphomidon (Shreelakshmi, 2008)

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 been suggested inhibition of 3β-HSD activity in adrenal cortex of rats fed with BHC. It has also been found that in rats fed with hexachlorocyclohexane caused inhibition of 17β-HSD and 3β-HSD and G-6PD in the testis as revealed by histochemical studies (Shivanandappa and Krishnakumari, 1982, 1983). Similarly, a organochlorine insecticide endosulfan, sodium arsenite, lithium chloride and lindane in rats shown to affect testicular androgenesis by inhibiting 3β-HSD and 17β-HSD activity (Shaw et al., 1979; Kerr and Sharpe, 1986; Ghosh et al., 1990; Chitra, 1999; Sujatha et al., 2001; Sarkar et al., 2003; Jana and Samata, 2005; Kuladip Jana et al., 2006). Therefore, the reduced activities of the important enzymes of steroidogenesis observed in the present study by indoxacarb may be due to.

- Impaired steroidogenesis in the Leydig cells and Sertoli cells of the seminiferous tubules (Blackshaw et al., 1970; Shivanandappa and Krishnakumari, 1990)
- 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 Jana et al., 2006)
- Affecting cytochrome P450 and G-6PD (Sidiqui et al., 1987; Stott et al., 1997; Siva Prasada Rao and Ramana Rao, 1978; Reddy et al., 1988)
- 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)
- Due to high affinity of binding of pesticide (Zarh et al., 2002) 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 indoxacarb on sodium-potassium adenosine triphosphatase (Na⁺-K⁺ATPase), magnesium adenosine triphosphatase (Mg²⁺ATPase) and calcium adenosine triphosphatase (Ca²⁺ATPase) activity of the testis and epididymis in mice

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; Desaiha, 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⁺-K⁺-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 Mg²⁺ and either K⁺ and Na⁺ ions alone to prostatic microsomal membrane preparations obtained from castrated adult rats have restored the Na⁺-K⁺ ATPase activity (Ahmed and Williams-Ashman, 1969). ATPases play important role in the ionic transport associated with secretory activities (Nalbandov, 1979). 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 vital role in the release and uptake of the biogenic amines in central nervous system (CNS) (Banks, 1965; Paton et al., 1971) and in nutrient transport (Lockall and Pfeffer, 1982). ATPase enzyme is associated with lipoprotein in the form of complex (Nakao et al., 1974). ATPase activity can be taken as meaningful index of cellular activity and forms a useful toxicological tool.

The results of the present investigation with graded dose exposure study revealed that, the activity of Na⁺-K⁺ATPase, Ca²⁺ATPase and Mg²⁺ATPase were decreased significantly in
the testis and epididymis in the mice treated with 18 and 24 mg indoxacarb. However, with 6 and 12 mg Indoxacarb treatment showed no significant change in the activity of ATPases. Similar results have been reported by intoxication of pesticides in testes (Ksheerasagar, 2005; Shreelakshmi, 2008). Similar findings have been also reported in rats treated with an organophosphate pesticide such as methyl parathion and parathion (Basha and Nayeemunnisa, 1993; Blasiak, 1995). It has been reported that carbamate pesticide thiram inhibited Na\(^+\)-K\(^+\)ATPase in rat testis (Mishra et al., 1998). Similarly, it has been reported in earlier studies in gonads and other tissues of pesticide treated rats (Seth et al., 1976; Srivastava et al., 1978).

Pesticides exert biological 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 Ca\(^{2+}\) spikes and regulation of Ca\(^{2+}\) 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\(^{2+}\)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; Dala et al., 1991). Therefore, the reduced activity of Na\(^+\)-K\(^+\)ATPase, Mg\(^{2+}\)ATPase and Ca\(^{2+}\)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 pumps in many tissues including brain (Guerra et al., 1987; Fraser and Swanson, 1994) which lead to impaired gonadal function.

g) Effect of indoxacarb on acid phosphatase (ACP) and alkaline phosphatase (AKP) activity of the testis and epididymis in mice

Acid phosphatase enzyme plays an important role in the process of cell metabolism, autolysis, differentiation and many related processes (Sugar, 1997). Dilatation of blood capillaries in between seminiferous tubules is the result of acid phosphatase enzyme activity. The increase, in acid phosphatase enzyme activity could be explained on the bases of enhancement of cell membrane permeability with disturbance in the transphosphorylation process as a result of cellular degeneration (Linder et al. 1988). AKP is a membrane bound enzyme found at bile pole of hepatocytes and also found in pinocytic vesicle and Golgi complex. It is present on all cell membranes where active transport occurs, and hydrolase and transphosphorylase in function. Decrease in AKP activity may be taken as index of hepatic parenchymal damage and hepatocytic necrosis (Onikienko, 1963). Inhibition of AKP reflects alterations in protein synthesis and uncoupling of oxidative phosphorylation (Verma et al., 1984). The decrease in AKP by stressors probably indicates an altered transport of phosphate (Engstrom, 1964) and an inhibitory effect on the cell growth and proliferation (Goldfischer et al., 1964).
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 18 and 24 mg indoxacarb treatment. However, 6 and 12 mg Indoxacarb treatment caused no significant change in testis and epididymis ACP and AKP activities. Similar results have been reported by intoxication of carbofuran (Ksheerasagar, 2005), and organophosphate pesticide phosphomidon (Shreelakshmi, 2008).

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) and hexachlorocyclohexane (HCH) treated mice (Ravinder et al., 1989). It has been reported that carbamate pesticides such as thiram and mancozeb exposure caused decrease in ACP and increase in AKP activities in testis of rats (Kacker et al., 1997; Mishra et al., 1998). Increased levels of AKP activity was also reported in methyl benzimicidal carbamate treated rats (Janardhan et al., 1987) and dichlorvos 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, sodium arsenite treated rats and mice (Dikshith et al., 1975; Janardhan and Sisodia, 1990; Dikshith et al., 1991; Bhatnagar and Soni, 1990; Prashanthi et al., 2006; Kuladip Jana et al., 2006; Narayana et al., 2006).

It has been reported that increase in acid phosphatase enzyme activity due was to intoxication of furadan on reproductive organs. MP-induced cell damage resulted in the release of ACP into the blood stream, hence reducing its level in the epididymis (Abraham and Wilfred, 2000), and this action of MP is similar to that of the pesticide quinalphos (Pant and Srivastava, 2003). 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 vacules and in spermatogonia, spermatocytes and spermatids in the testis of the rats (Tice and Bannett, 1963) and mice (Dieteri, 1966; Porier, 1975). 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). 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 Bernett, 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 indoxacarb on oxidative stress parameters of the testis in 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. 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. 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. Other sources include monoxygenases 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.

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 cardio toxicity. Oxidative stress, generated by xenobiotics, induces disturbances in antioxidant enzyme systems (Gabbianelli 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 et al., 1999). The effects of organophosphate insecticides on fish revealed that besides acetylcholinesterase inhibition, there were changes characteristic of oxidative stress (Malkovics 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 et al., 2001).

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).

**Glutathione (GSH) and thiobarbutaric acid (TBARS)**

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). 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 sulphydryl compound that serves as an effective reductant and a nucleophile that interacts with numerous electrophilic and oxidizing compounds.

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 are 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. Plasmatic membranes of spermatozoa contain high concentrations of polyunsaturated fatty acids and therefore are highly sensitive to oxidative stress.

In the present graded dose exposure study, the mice treated with 18 and 24 mg indoxacarb caused decrease in GSH and increase in the TBARS in testis. However, 6 and 12 mg indoxacarb treatments caused no change in GSH and TBARS levels in testis of mice.

It is 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, we have previously reported the development of a hydroperoxides model of oxidative stress (OS) in testis and examined the correlation among LPO in testis, DNA damage, and genotoxic implications (Rajeshkumar and Muralidhara, 1999; Rajeshkumar et al., 2002). High doses of fluoride (F) have been found to interfere with the reproductive system of animals and induce oxidative stress (Ghosh et al., 2002; Das et al., 2005; Krechniak and Inkielewicz, 2005; Chlubek 2003) commonly observed effects of fluorine treated animals. It has been reported that oxidative stress in the testes of male mice exposed to different doses of F (as NaF), in agreement with
other reports. (Zhang et al., 2006, 2006; Chinoy et al., 2005) Because of its dense negative charge, F has a very strong hydrogen bonding capacity and is prone to bind various antioxidants and anti-oxidation enzymes. It thus interferes with many metabolic processes, including promotion of reactive free radical oxygen species that attack cell membranes and even lead to cell apoptosis (Ge et al., 2006). It has also been confirmed that germ cells are more susceptible to oxidative stress than somatic cells (Zhang et al., 2006). Therefore, it is plausible that F in the testis of mice causes an increase in MDA and a decrease in the activity of SOD and GSH-Px. 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). 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. Di (2-ethylhexyl) phthalate (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. Recently it has 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 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 organic hydrogen peroxide and tertiary butyl hydrogen peroxide has 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). Hydrogen peroxide (HP) treatment induced a significant increase in the lipid peroxidation and enhanced ROS generation in testis after 1- and 2-week exposure, clearly suggesting their potential to induce significant oxidative stress in the reproductive milieu of rats. These results are consistent with the earlier data on HP induced oxidative damage in rat liver (Younes and Weiss, 1990) and in testis of mice (Rajeshkumar et al., 2002; Kaur et al., 2006) and rats (Li et al., 2006). It revealed that, although only a moderate increase in LPO was evident after 1-week exposure, the elevation was marked at the end of the treatment, clearly suggesting the cumulative effect of HP. 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 the 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. In the present study the reason for increased MDA level in testes under the influence of indoxacarb treatment in mice might caused due to the conjugation of indoxacarb 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.

In the present study, the decreased levels of GSH and increased level of TBARS in the testis of mice under the influence of indoxacarb treatment may be due to oxidative stress to different doses of indoxacarb.

a) The indoxacarb is a fluorinated compound prone to bind various antioxidants and antioxidation enzymes as it has been observed oxidative stress in the testes of mice exposed to different doses of F(NaF) (Chinoy et al., 2005; Zhang et al., 2006, 2006).

b) Many of the metabolites of pesticides are also conjugated with glutathione, causing depletion of the glutathione reserve (Roy Ananya et al., 2005) this might be the mechanism underlying the decrease in glutathione levels in our study.
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.

Ascorbic acid and protein carbonyl

In the present graded dose exposure study, the male mice treated with 18 and 24 mg indoxacarb caused decrease in ascorbic acid and increase in the protein carbonyl in testis. However, 6 and 12 mg indoxacarb treatments caused no change in ascorbic acid and protein carbonyl of levels in testis of male mice.

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; Bensousan et al., 1998). Ascorbic acid is 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 (Gualiquil et al., 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).
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. 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).

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 (Narayana et al., 2005a; Matthew et al., 1992). 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).

Organic hydrogen peroxide (HP) such as Tertbutyl 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). Further 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 evidence of 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). 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 (Thimmappa and Muralidhara, 2007). 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 both ascorbic acid (Thimmappa and Muralidhara, 2007).

In the present study, the decreased level of ascorbic acid and increased level of protein carbonyl in testis of mice are under the influence of indoxacarb 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) The decrease in the level of ascorbic acid tends to oxidative stress as it has been reported that ascorbic acid is a potent water-soluble antioxidant in biological fluids, scavenging physiologically relevant reactive oxygen and nitrogen species, there by preventing oxidative damage to vital biomolecules (Thyagaraju and Muralidhara, 2008).
**Super oxide dismutase (SOD) and catalase (CAT)**

Reactive oxygen species (ROS) must be continuously inactivated to keep only a small amount necessary to maintain normal cell function. Seminal plasma is endowed with an array of antioxidants to protect spermatozoa against oxidants (Sikka, *et al.*, 1996; Alvarez, *et al.*, 1995; Armstrong, *et al.*, 1998). 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 isoymes, and catalase have a significant role. SOD spontaneously dismutates \((O_2^-)\) anion to form \(O_2\) and \(H_2O_2\), while catalase converts \(H_2O_2\) to \(O_2\) and \(H_2O\).

\[
\text{SOD} \\
2(O_2^-) + 2H \xrightarrow{} H_2O_2 + O_2
\]

\[
\text{Catalase} \\
H_2O_2 \xrightarrow{} H_2O + 1/2O_2
\]

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, the male mice treated with 18 and 24 mg indoxacarb caused increase in the enzyme activity of SOD and Catalase in testis. However, 6 and 12 mg indoxacarb treatments caused no change in enzyme activity of SOD and Catalase in testis.

There are evidences of oxidative stress in the testes of male mice exposed to different doses of F (as NaF) (Zhang *et al.*, 2006, 2006; Chinoy *et al.*, 2005). Because of its dense negative charge, F has a very strong hydrogen bonding capacity and is prone to bind various antioxidants and anti-oxidation enzymes. It thus interferes with many metabolic processes, including promotion of reactive free radical oxygen species that attack cell membranes and even...
lead to cell apoptosis (Ge et al., 2006). It has also been confirmed that germ cells are more susceptible to oxidative stress than somatic cells (Zhang et al., 2006). Therefore it is plausible that F in the testis of mice causes an increase in MDA and a decrease in the activity of SOD and Glutathione peroxidase (GSH-Px). The present findings revealed that, with increase in high dose exposure to indoxacarb showed increase in the activity of catalase and SOD. In the present model, HP treatment significantly enhanced the activities of various antioxidant enzymes in testis. The concomitant increase in the activities of catalase (CAT) and Glutathione peroxidase (GPX) suggests that HP may increase the level of hydrogen peroxide, the substrates for these enzymes. It has been reported that DEHP enhanced the generation of ROS in testicular cells Cu/Zn-SOD and catalase revealed were increased (Emiko et al., 2002). It is reported that lindane causes oxidative stress in the testes and increases catalase and SOD activity might be due to higher levels of SOD and catalase following adaptation might have protected the testes from more severe injury due to oxidative stress. Recently 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) 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 obtained by Latchoumycandane et al.,(2002) in testes of rats after oral administration of methoxychlor in the daily doses of 50, 100, or 200 mg/kg body weight for 1, 4 or 7 days.

In the present study, increased levels of catalase and SOD in testes of mice are under the influence of indoxacarb treatment may be due to

a) Scavenging of superoxide and hydrogen peroxide produced due to intoxication of indoxacarb.

b) Decreases in glutathione and protein-binding sulfhydryl groups, which causes an increase in reactive oxygen species like hydrogen peroxide, hydroxyl radicals, and superoxide radicals, which increase lipid peroxidation, change intercellular stability, damage deoxyribonucleic acid (DNA) and membranes, and cell death (Stohs, 2001).
Glutathione-s-transferase (GST)

In the present graded dose exposure study, the male mice treated with 18 and 24 mg indoxacarb caused increase in the enzyme activity of glutathione s-transferase in testis. However, 6 and 12 mg indoxacarb treatments caused no change in enzyme activity of glutathione s-transferase in the testis of mice.

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 P<sub>450</sub> (CYP), catalyse the incorporation of a functional group (-OH, -NH<sub>2</sub>, -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).

Increase in the activities of catalase (CAT) and glutathione peroxidase (GPX) suggests that HP may increase the level of hydrogen peroxide, the substrates for these enzymes. GSTs are a group of primary phase II detoxification enzymes that provide protection against products of oxidative stress whose abundance and protective role in germ cells has been adequately demonstrated (Aravinda et al., 1995; Hayes and Guilford, 1995; Rao and Shaha, 2000). It has been shown that increase in the activity of GST due to organochlorine contaminants indeed, GST participates in pollutant detoxification by adding a GSH-group to xenobiotics or their metabolite, so they become more water-soluble and, are excreted more easily (Machala, et al., 1998; Moorhouse and Casida, 1992). The activity of GST in-vitro has been shown (Rajeshkumar and Muralidhara, 2002; Hemchand and Shaha, 2003), suggesting its vital role under HP intoxication was markedly enhanced; we speculate they play a major role in the regulation of oxidative stress products in this model (Thimmappa and Muralidhara, 2007).

In the present study the reason for increased activity of glutathione s-transferase under the influence of indoxacarb treatment in mice testes may be due to ROS produced by the
indoxacarb or to detoxify the pesticide or in order to eliminate the pesticide from the body by conjugation with the GSH to become more water soluble.

i) Agarose gel electrophoresis of cellular DNA in the testis of mice after exposure to indoxacarb.

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 12, 18 and 24 mg of indoxacarb for 30 days showed a smear of DNA indicates of the 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 (Stachlewitz et al., 1999; Tsutsu et al., 1997).
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 indoxacarb showed a smear of DNA indicates necrosis cell death of the testes in mice may be due to random cleavage of base pair during the necrotic process (Emanuela et al., 1995).
SUMMARY AND CONCLUSION

The present study was aimed to elucidate the graded doses exposure of indoxacarb on testis and epididymis, biochemical contents and oxidative stress parameters (GSH, TBARS, protein carbonyl, ascorbic acid, catalase, super oxide dismutase, and glutathione-s-transferase) in mice.

1. Treatment with 18 and 24 mg indoxacarb caused significant decrease in the weight of testes and epididymides. However, treatment with 6 and 12 mg indoxacarb treatment caused no significant change in the weight of testes and epididymides, when compared with those of the corresponding parameters of the control mice.

2. There was a significant decrease in the number and diameter of spermatogenic and Leydig cells with 18 and 24 mg indoxacarb treatment. However, treatment with 6 and 12 mg indoxacarb 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.

3. Mice treated with 18 and 24 mg indoxacarb caused significant decrease in the level of DNA, RNA, protein and glycogen, whereas cholesterol content was increased significantly in testis and epididymis. In the mice treated with 6 and 12 mg indoxacarb caused no significant change in the level of the biochemical contents of testis. When compared with those of the corresponding parameters of the control mice.

4. Mice treated with 18 and 24 mg indoxacarb showed significant decrease in the activity of SDH, 3β-HSD, 17β-HSD, Na⁺-K⁺ATPase, Mg²⁺ATPase, Ca²⁺ATPase and ACP in testis. However, the activity of the LDH and AKP were increased significantly. In the mice treated with 6 and 12 mg indoxacarb caused no significant change in the activity of enzymes in testis, when compared with those of the corresponding parameters of the control mice.

5. Mice treated with 18 and 24 mg indoxacarb showed significant decrease in the activity of SDH, Na⁺-K⁺ATPase, Mg²⁺ATPase, Ca²⁺ATPase and ACP in epididymis. However,
the activity of the LDH and AKP were increased significantly. In the mice treated with 6 and 12 mg indoxacarb caused no significant change in the activity of enzymes in epididymis, when compared with those of the corresponding parameters of the control mice.

6. Study on oxidative stress parameters (antioxidants and oxidative stress byproducts) of the testis revealed that, in the mice treated with 18 and 24 mg indoxacarb caused significant decrease in the level of GSH and Ascorbic acid contents, whereas TBARS, protein carbonyl content was increased significantly. In the mice treated with 6 and 12 mg indoxacarb, there was no significant change in the level of the oxidative stress parameters (antioxidants and oxidative stress byproducts) of testis when compared with those of the corresponding parameters of the control mice.

7. Study on the activity of oxidative stress enzymes in the testis revealed that, in the mice treated with 18 and 24 mg indoxacarb showed significant increase in the activity of catalase, SOD, and Glutathione s-transferase. In the mice treated with 6 and 12 mg indoxacarb caused no significant change in the activity of enzymes, when compared with those of the corresponding parameters of the control mice.

8. The results of the DNA gel electrophoresis analysis in testes of the control and with 12, 18 and 24 mg/kg body weight indoxacarb treated mice revealed that the DNA band was observed in lane 1 of the cells in the testes of control mice. However, lane 2, 3 and 4 showed smear of DNA in the tissue of the testes in mice treated with 12, 18 and 24 mg/kg body weight of indoxacarb for 30 days respectively indicates necrosis of the cells.

In conclusion, the present results suggests that indoxacarb is a reproductive toxicant in males affecting weight of testes and epididymides, number and diameter of spermatogenic and Leydig cells, biochemical contents, oxidative stress parameters. 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. It appears, therefore, that indoxacarb treatment produces degenerative changes in spermatogenic cells and inhibits
androgen production acting primarily at the level of hypothalamo-hypophysial gonadal axis. The oxidative stress parameters like protein carbonyl and TBARS were increased due to stress and antioxidants like ascorbic acid were decreased due to oxidative stress and antioxidant enzymes are increased due to stress.
Table 1.1 Testes and Epididymides weight in mice following oral exposure to indoxacarb

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/ kg/day)</th>
<th>Testes (mg)</th>
<th>Epididymis (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>830.8 ± 1.00</td>
<td>390.0 ± 2.00</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>819.6 ± 1.53</td>
<td>345.4 ± 2.02</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>799.4 ± 1.25</td>
<td>290.3 ± 3.07</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>725.4 ± 1.32*</td>
<td>279.0 ± 2.90*</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>650.4 ± 2.91*</td>
<td>240.4 ± 0.37*</td>
</tr>
</tbody>
</table>

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

Table 1.2 Effect of indoxacarb on number and diameter of spermatogenic and Leydig cells in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/ kg/day)</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>70.47 ± 3.34</td>
<td>95.39 ± 3.12</td>
<td>103.51 ± 4.56</td>
<td>174.40 ± 2.41</td>
<td>57.61 ± 2.31</td>
<td>8.59 ± 0.09</td>
<td>8.98 ± 0.34</td>
<td>7.58 ± 0.28</td>
<td>5.91 ± 0.36</td>
<td>8.94 ± 0.38</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>65.26 ± 3.10</td>
<td>91.10 ± 3.14</td>
<td>96.10 ± 4.45</td>
<td>169.30 ± 3.21</td>
<td>51.94 ± 2.28</td>
<td>7.86 ± 0.11</td>
<td>8.15 ± 0.28</td>
<td>7.04 ± 0.34</td>
<td>5.21 ± 0.34</td>
<td>8.68 ± 0.28</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>60.70 ± 3.34</td>
<td>87.30 ± 2.41</td>
<td>93.63 ± 4.00</td>
<td>164.40 ± 3.31</td>
<td>45.94 ± 2.83</td>
<td>6.80 ± 0.31</td>
<td>7.54 ± 0.18</td>
<td>6.55 ± 0.22</td>
<td>4.45 ± 0.28</td>
<td>7.94 ± 0.31</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>59.18 ± 2.89*</td>
<td>85.20 ± 2.81*</td>
<td>90.46 ± 3.13*</td>
<td>160.02 ± 2.78*</td>
<td>38.51 ± 2.19*</td>
<td>5.90 ± 0.21*</td>
<td>6.75 ± 0.23*</td>
<td>5.95 ± 0.31*</td>
<td>3.61 ± 0.21*</td>
<td>7.13 ± 0.27*</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>56.00 ± 3.57*</td>
<td>83.10 ± 3.47*</td>
<td>89.10 ± 3.16*</td>
<td>158.01 ± 3.15*</td>
<td>30.18 ± 3.05*</td>
<td>4.70 ± 0.26*</td>
<td>5.83 ± 0.31*</td>
<td>5.08 ± 0.35*</td>
<td>2.88 ± 0.24*</td>
<td>6.87 ± 0.34*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 10 animals * Significant P ≤ 0.05 compared to control
Table 1.3 Effect on biochemical contents of the testis in mice after exposure to indoxacarb

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/day)</th>
<th>DNA</th>
<th>RNA</th>
<th>Protein</th>
<th>Glycogen</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>2.30 ± 0.10</td>
<td>5.10 ± 0.40</td>
<td>160.01 ± 3.50</td>
<td>6.50 ± 0.34</td>
<td>9.25 ± 0.33</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>2.19 ± 0.07</td>
<td>4.67 ± 0.30</td>
<td>156.35 ± 3.20</td>
<td>5.99 ± 0.50</td>
<td>9.71 ± 0.30</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>2.09 ± 0.06</td>
<td>4.35 ± 0.04</td>
<td>150.21 ± 2.21</td>
<td>4.80 ± 0.5</td>
<td>10.46 ± 0.37</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>1.99 ± 0.05*</td>
<td>3.58 ± 0.08*</td>
<td>140.05 ± 2.28*</td>
<td>4.20 ± 0.41*</td>
<td>11.01 ± 0.39*</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>1.85 ± 0.05*</td>
<td>3.43 ± 0.04*</td>
<td>130.04 ± 2.23*</td>
<td>3.97 ± 0.32*</td>
<td>12.20 ± 0.51*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 10 animals. * Significant P ≤ 0.05 compared to control.

Table 1.4 Effect on testis dehydrogenase and phosphatase enzymes activity in mice after exposure to indoxacarb

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/day)</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>8.50 ± 0.15</td>
<td>11.90 ± 0.20</td>
<td>0.37 ± 0.02</td>
<td>0.90 ± 0.08</td>
<td>7.70 ± 0.20</td>
<td>8.79 ± 0.50</td>
<td>6.20 ± 0.32</td>
<td>18.82 ± 0.50</td>
<td>13.98 ± 0.40</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>8.68 ± 0.17</td>
<td>10.50 ± 0.40</td>
<td>0.35 ± 0.01</td>
<td>0.85 ± 0.05</td>
<td>7.48 ± 0.41</td>
<td>8.18 ± 0.51</td>
<td>5.98 ± 0.22</td>
<td>16.34 ± 0.58</td>
<td>14.50 ± 0.20</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>9.18 ± 0.30</td>
<td>9.20 ± 0.35</td>
<td>0.34 ± 0.01</td>
<td>0.79 ± 0.06</td>
<td>6.52 ± 0.40</td>
<td>7.26 ± 0.50</td>
<td>5.90 ± 0.10</td>
<td>15.98 ± 0.40</td>
<td>15.01 ± 0.30</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>9.97 ± 0.20*</td>
<td>8.15 ± 0.38*</td>
<td>0.30 ± 0.02*</td>
<td>0.73 ± 0.08*</td>
<td>5.48 ± 0.40*</td>
<td>6.30 ± 0.10*</td>
<td>5.70 ± 0.15*</td>
<td>15.25 ± 0.35*</td>
<td>15.35 ± 0.20*</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>10.31 ± 0.18*</td>
<td>7.07 ± 0.40*</td>
<td>0.28 ± 0.01*</td>
<td>0.65 ± 0.05*</td>
<td>4.58 ± 0.45*</td>
<td>6.20 ± 0.05*</td>
<td>5.35 ± 0.22*</td>
<td>15.07 ± 0.15*</td>
<td>15.74 ± 0.18*</td>
</tr>
</tbody>
</table>

\(a\) 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  
Values are mean ± SEM of 10 animals. * Significant P ≤ 0.05 compared to control.
Table 1.5 Oxidative stress parameters of the testis in mice after exposure to indoxacarb

<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>1.75 ± 0.20</td>
<td>300 ± 25</td>
<td>0.65 ± 0.25</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>1.70 ± 0.10</td>
<td>280 ± 10</td>
<td>0.75 ± 0.10</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>1.55 ± 0.05</td>
<td>250 ± 15</td>
<td>0.95 ± 0.15</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>1.43 ± 0.08*</td>
<td>200 ± 30*</td>
<td>1.28 ± 0.13*</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>1.30 ± 0.10*</td>
<td>172 ± 15*</td>
<td>1.55 ± 0.18*</td>
</tr>
</tbody>
</table>

\(a\) \(\mu\)mole of glutathione(GSH)/mg protein  
\(b\) ngm of ascorbic acid  
\(c\) nmole of thiobarbaturic acid(TBARS)/gm protein  
\(d\) nmole of protein carbonyl/mg protein  
Values are mean ± SEM of 10 animals.

\(e\) \(\mu\)mole of H\(_2\)O\(_2\)  
\(f\) super oxide dismutase(SOD) unit/mg protein  
\(g\) Glutathione-s-transferase(GST)  
\(\mu\)mole/min/mg protein  
* Significant \(P \leq 0.05\) compared control.
Table 1.6 Biochemical contents of epididymis in mice after exposure to indoxacarb

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/day)</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>3.10±0.14</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>2.95±0.16</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>2.50±0.14</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>2.20±0.18</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>1.90±0.14*</td>
</tr>
</tbody>
</table>

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

Table 1.7 Effect on epididymis dehydrogenase and phosphatase enzymes activity in mice after exposure to indoxacarb

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/day)</th>
<th>Enzyme activity µmoles/ min/ g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LDH&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>13.45±0.31</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>13.98±0.26</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>14.93±0.25</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>16.09±0.24*</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>17.27±0.28*</td>
</tr>
</tbody>
</table>

<sup>a</sup> µmoles of pyruvate formed/ min/ g tissue  
<sup>b</sup> µmoles of formazan formed/ min/ g tissue  
<sup>c</sup> µmoles of NAD converted to NADH/ min/ g tissue  
<sup>d</sup> µmoles of NADPH converted to NADP/ min/ g tissue  
<sup>e</sup> µmoles of inorganic phosphorus formed/ min/ g tissue  
<sup>f</sup> µmoles of p-nitrophenyl formed/ min/ g tissue  

Values are mean ± SEM of 10 animals  * Significant P ≤ 0.05 compared to control
Graph 1.1. Testes and epididymides weight in mice following oral exposure to indoxacarb

Graph 1.2. Effect of indoxacarb on number of spermatogenic and Leydig cells in mice
Graph 1.3. Effect of indoxacarb on diameter of spermatogenic and Leydig cells in mice

Graph 1.4. Biochemical contents of the testis in mice after exposure to indoxacarb
Graph 1.5. Effect on testis dehydrogenase and phosphatase enzymes activity in mice after exposure to indoxacarb.

Graph 1.6. Oxidative stress parameters of the testis in mice after exposure to indoxacarb.

Graph 1.6 Oxidative stress parameters of the testis in mice after exposure to indoxacarb

Graph 1.5. Effect on testis dehydrogenase and phosphatase enzymes activity in mice after exposure to indoxacarb

Graph 1.6 Oxidative stress parameters of the testis in mice after exposure to indoxacarb
Graph 1.7 Oxidative stress parameters of the testis in mice after exposure to indoxacarb

Graph 1.8. Biochemical contents of epididymis in mice after exposure to indoxacarb
Graph 1.9. Effect on epididymis dehydrogenase and phosphatase enzymes activity in mice after exposure to indoxacarb.

<table>
<thead>
<tr>
<th>Treatment (mg/kg/day)</th>
<th>LDH</th>
<th>SDH</th>
<th>Na+ K+ ATPase</th>
<th>Ca++ ATPase</th>
<th>Mg ++ ATPase</th>
<th>ACP</th>
<th>AKP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Significant