CHAPTER - III

INDOXACARB INDUCES LIVER TOXICITY, BIOCHEMICAL CHANGES AND OXIDATIVE STRESS PARAMETERS IN ALBINO MICE
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V SUMMARY AND CONCLUSION
INTRODUCTION

Pesticides are used for the welfare of human beings but by the time, they will challenge us by showing their toxicity. We can be exposed directly or indirectly through food chain. Indiscriminate use of pesticides is on increase. India is one of the largest user of agricultural pesticides such as organophosphates, carbamates etc. Pesticides are toxic compounds to all living organisms however effects vary from species to species. But excessive use of these pesticides creates many problems to all of us. These days, synthetic chemical pesticides are in practice because of their active and best results. But their excessive use causes serious damage to ecosystem - terrestrial as well as aquatic and consequently the flora and fauna of the surrounding. Liver is a target organ and primary site of detoxification and is generally the major site of intense metabolism and is therefore prone to various disorders as a consequence of exposure to the toxins of extrinsic as well as intrinsic forms. Liver plays important role in metabolism to maintain energy level and structural stability of body (Guyton and Hall, 2002). It is also a site of biotransformation by which a toxic compound has been transformed in less harmful form to reduce toxicity (Hodgson, 2004).

Carbamates have been reported to have high mammalian toxicity, and the main target organs are brain, liver, skeletal muscles, and heart (Gupta, 1994; Risher et al., 1987). Their primary mechanism of toxicity is the reversible inhibition of serine group of acetylcholinesterase via carbamoylation at the nerve terminals by organophosphorus and carbamate pesticides (Fukuto, 1990). In addition to inhibition of acetylcholinesterase activity, carbamates affect many nonspecific sites. Liver is the primary site involved in the metabolism of various drugs and chemicals including carbamates (Pineiro-Carrero and Pineiro, 2004). In addition, kidney and reproductive functions have been reported to be adversely affected with carbamate exposure to rats (Kareem et al., 2007, Bindali and Kaliwal, 2002). Cholesterol and triglyceride levels have been reported to be elevated in liver after carbofuran exposure (Gupta et al., 1994). Another anticholinesterase compound, chloropyriphos has been reported to cause hyperlipidemia in rats (Slotkin et al., 2005). The mechanism of the toxic action of dimethoate (similar to other OP compounds) is one of the inhibiting acetylcholinesterase activity. The 8-week rat liver assay uses the development of altered foci of hepatocytes (AFH) as endpoint, which express the placental form of the enzyme glutathione s-transferase (GST-P+foci) and have been considered as early indicators of the rat liver carcinogenic process due to toxicity.
induced by the diuron (Tsuda et al., 2003; Ito et al., 2003). Monocrotophos (MCP) into mice gave high incidence of structural and numerical chromosomal aberrations in both somatic and germ cells of males and liver and embryos of pregnant females (Zahran et al., 2005). 2, 4-dichlorophenoxyacetic acid (2, 4-D) is a selective herbicide, hepatotoxic effects of 2,4-D have been well documented (Barenekow et al., 2000; Charles et al., 2001; Osaki et al., 2001).

Organophosphates (OP) are a group of compounds that have historically been used as pesticides as well as chemical warfare agents. These compounds are potent acetylcholinesterase inhibitors that have a profound effect on the nervous system, liver, kidneys and salivary glands (Agency for toxic substances and disease registry (ATSDR), 2001; Fazekas, 1971) (Vale, 1998) (Butler and Murray, 1993). Owing to the significant role played by the liver cells in the detoxification of various xenobiotics, liver injury gained wide spread of scientific and public attractions. Liver is among the first organs to be affected by different chemicals gained entry into animal body regardless the route of administration. The toxic compounds interfere with key enzymatic processes that are concerned with normal physiology of the animal. This includes carbohydrates, proteins and lipid metabolism. Biochemical changes occur before tissue pathological symptoms appear.

Further exposure of pesticides induces the Oxidative stress which induces the oxidative stress enzymes to compensate the stress induced by these pesticides (al-Bayati, et al., 1988; Hermansky et al., 1988; Gupta, et al., 1994; Bagchi et al., 1995; Hassoun and Stohs, 1996; Junqueira et al., 1997; Bachowski et al., 1998; Samanta et al., 1999; Sahoo et al., 2000; Faraone-Mennella et al., 2003; Abdollahi et al., 2004; Goel et al., 2005). Free radicals are continuously produced during normal physiologic events and attack macromolecules including proteins, DNA, and lipids, causing tissue injury. The body has developed major antioxidant defense mechanisms for the removal of free radicals include glutathione, superoxide dismutase (SOD), glutathione reductase, peroxidase, glutathione-s-transferase and catalase (CAT) enzymes (Betteridge, 2000; Halliwell, 1994). Reactive oxygen species (ROS) generated by exogenous and endogenous factors, cellular metabolism, etc. (Grishko et al., 2005). Dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) and polychlorinated biphenyls, which act as hepatocarcinogens in rodents, induced CYP1A1/2 and 1B1 and increased the ROS production in the rodent liver, and that ROS may play an important role in a variety of diseases (Coumoul et al., 2001; Hatanaka et al., 2001; Santostefano et al., 1999; Shimada et al., 1996; Van Birgelen et al., 1995; Walker et
al., 1999). Therefore, the present investigation was undertaken to study the effect of indoxacarb on liver biochemical contents, enzymes activity and oxidative stress parameters of the liver in albino mice.
MATERIALS AND METHODS

Normal female and male mice aged 90-120 days old weighing 25-35 g were used for the experiments. The mice were maintained as mentioned in chapter-I. Mice were randomly divided into five groups of 20 (10 male and 10 female) mice in each group. Experiments were carried out as follows:

A 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 histology, biochemical contents and oxidative stress parameters (antioxidants, oxidative stress byproducts and oxidative stress enzymes) of the liver in albino mice. Distilled water treated mice were served as controls. Individual animal weights were recorded weekly throughout the experiment.

All mice were autopsied on 31st day after 24 hours of last treatment the liver was dissected out freed from adherent tissues and weighed to the nearest milligram. Liver was fixed in Bouin's fluid for histologic studies, estimation of biochemical contents such as DNA, RNA, protein, glycogen, cholesterol and activity of enzymes such as SDH, LDH, Na⁺-K⁺ ATPase, Ca²⁺ATPase, Mg²⁺ATPase, ACP and AKP were carried out as per the methods mentioned in chapter-I. The ASAT and ALAT, estimations were performed as per the method described by Yatzidis (1960).

Estimation of aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activity

ASAT and ALAT activity in tissues were estimated by the method followed by Yatzidis (1960).

Principle: In transamination an amino group is transferred from an alpha amino acid to an alpha keto group to form a different alpha amino acid and keto acid. The oxaloacetate in the presence of aniline citrate is converted into pyruvate, in both the cases pyruvate reacts with 2, 4-DNPH and form a red coloured complex dinitrophenyl hydrazone which can be measured at 500 nm.
The 10% of tissue homogenate (w/v) was prepared in ice cold 0.8 M sucrose and centrifuged at 10,000 rpm for 10 minutes at 4°C. The 1 ml of supernatant was used as enzyme source and to that 0.5 ml of ASAT substrate (2.66 gm of DL-aspartic acid, 30 mg of α-ketoglutaric acid, 2 gm of dipotassium hydrogen phosphate were dissolved in 80 ml of distilled water and pH adjusted to 7.4 with 0.75 M NaOH and solution is made up to 100 ml with distilled water. Few drops of 1% chloroform was added as preservative) was added. To another set of test tubes 1 ml enzyme source and 0.5 ml of ALAT substrate (1.78 g of DL-alanine, 30 mg of α-ketoglutaric acid and 2 g of dipotassium hydrogen phosphate were dissolved in 80 ml of distilled water. The pH was adjusted to 7.4 with 0.75 M NaOH and solution made up to 100 ml with distilled water. Few drops of 1% chloroform were added as a preservative) was added and were placed in a water bath for 60 minutes at 37°C. The tubes were held at room temperature and 0.5 ml of Aniline citrate (5 gm citric acid, 5 ml distilled water and 5 ml of redistilled aniline were added. This solution was diluted to 1:5 with distilled water just before use) was added to stop the enzymatic reaction and 0.5 ml of DNPH (40 mg of 2,4-dinitrophenyl hydrazine, 5 ml of concentrated HCl was added and solution made up to 100 ml with distilled water) was added immediately and the contents were mixed vigorously. Exactly 5 minutes after the addition of DNPH, 3 ml of NaOH (0.75 M) was added and the contents were mixed by inversion. Simultaneously blank and standards were prepared. The color produced was measured after 30 minutes at 500 nm. The enzyme activity expressed as μmoles pyruvate formed / min/ g of tissue.

Statistical analysis

Statistical calculations were carried out as per the method mentioned in Chapter-I.
OBSERVATIONS

a) Effect on liver biochemical contents (DNA, RNA, protein, glycogen and cholesterol) in female mice after exposure to indoxacarb (Table 3.1; Graph 3.1)

In the control female mice the level of DNA, RNA, protein, glycogen and cholesterol was 1.79, 3.60, 188.8, 6.30 and 10.01 µg respectively in the liver. In the female mice treated with 6 mg indoxacarb, the level of DNA, RNA, protein, glycogen and cholesterol was 1.71, 3.42, 180.00, 5.86 and 10.28 µg respectively in the liver. There was no significant change in the level of biochemical contents when compared with those of the corresponding parameters of the control mice.

In the female mice treated with 12 mg indoxacarb the level of DNA, RNA, protein, glycogen and cholesterol was 1.6, 3.19, 165.6, 5.07 and 11.39 µg respectively in the liver. There was no significant change in the level of biochemical contents when compared with those of the corresponding parameters of the control mice.

In the female mice treated with 18 mg indoxacarb, the level of DNA, RNA, protein, glycogen and cholesterol was 1.55, 3.04, 156.00, 3.85 and 12.46 µg respectively in the liver. However, the levels of DNA, RNA, protein and glycogen were decreased significantly, whereas cholesterol level was increased significantly when compared with those of the corresponding parameters of the control mice.

In the female mice treated with 24 mg indoxacarb, the level of DNA, RNA, protein, glycogen and cholesterol was 1.49, 2.60, 140.40, 2.45 and 13.85 µg respectively. There was a significant decrease in the level of DNA, RNA, protein and glycogen. However, the level of cholesterol was increased significantly when compared with those of the corresponding parameters of the control mice.

Results of the present study on biochemical contents of the liver in female mice revealed that the treatment with 6 and 12 mg indoxacarb showed no significant change in the level of biochemical contents whereas, treatment with 18 and 24 mg indoxacarb caused significant decrease in the level of DNA, RNA, protein and glycogen and significant increase in the level of cholesterol, when compared with those of the corresponding parameters of the control mice.
b) Effect on liver biochemical contents (DNA, RNA, protein, glycogen and cholesterol) in male mice after exposure to indoxacarb (Table 3.2; Graph 3.2)

In the control male mice the level of DNA, RNA, protein, glycogen and cholesterol was 1.96, 3.80, 215.40, 6.86 and 10.54 μg respectively in the liver. In the male mice treated with 6 mg indoxacarb, the level of DNA, RNA, protein, glycogen and cholesterol was 1.79, 3.47, 211.75, 6.80 and 11.42 μg respectively in the liver. There was no significant change in the level of biochemical contents when compared with those of the corresponding parameters of the control mice.

In the male mice treated with 12 mg indoxacarb the level of DNA, RNA, protein, glycogen and cholesterol was 1.63, 3.22, 189.7, 5.95 and 12.07 μg respectively in the liver. There was no significant change in the level of biochemical contents when compared with those of the corresponding parameters of the control mice.

In the male mice treated with 18 mg indoxacarb the level of DNA, RNA, protein, glycogen and cholesterol was 1.44, 2.88, 174.45, 5.04 and 12.95 μg respectively in the liver. There was a significant decrease in the level of DNA, RNA, protein and glycogen except, cholesterol where it was increased significantly when compared with those of the corresponding parameters of the control mice.

In the male mice treated with 24 mg indoxacarb, the level of DNA, RNA, protein, glycogen and cholesterol 1.34, 2.60, 168.45, 4.20 and 13.59 μg respectively in the liver. There was a significant decrease in the level of DNA, RNA, protein and glycogen contents. However, the level of cholesterol was increased significantly when compared with those of the corresponding parameters of the control mice.

Results of the present study on biochemical contents of the liver in male mice revealed that treatment with 18 and 24 mg indoxacarb caused significant decrease in the level of DNA, RNA, protein, glycogen and significant increase in the level of cholesterol. However, treatment with 6 and 12 mg indoxacarb caused no significant change in the level of biochemical contents, when compared with those of the corresponding parameters of the control mice.
c) Effect on liver dehydrogenase (LDH and SDH), aminotransferase (ASAT and ALAT) and phosphatase (ATPases, ACP and AKP) enzymes activity in female mice after exposure to indoxacarb (Table 3.3; Graph 3.3)

In the female control mice, the activity of the LDH, SDH, ASAT, ALAT, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase, ACP and AKP enzymes was 12.73, 13.10, 15.99, 15.54, 4.08, 6.93, 3.78, 14.40 and 15.70 μmoles respectively in the liver. Treatment with 6 mg indoxacarb, the activity of the LDH, SDH, ASAT, ALAT, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase, ACP and AKP was 13.01, 12.50, 16.44, 16.03, 3.55, 6.25, 3.28, 14.06 and 16.35 μmoles respectively in the liver. There was no significant change in the activity of the enzymes when compared with that of the control mice.

In the female mice treated with 12 mg indoxacarb the activity of LDH, SDH, ASAT, ALAT, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase, ACP and AKP enzymes was 13.73, 11.73, 16.99, 16.76, 3.47, 5.61, 2.76, 13.34 and 17.20 μmoles respectively in the liver. There was no significant change in the activity of the enzymes when compared with that of the control mice.

In the female mice treated with 18 mg indoxacarb, the activity of LDH, SDH, ASAT, ALAT, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase, ACP and AKP was 14.60, 11.03, 17.67, 17.24, 2.99, 4.91, 2.30, 12.25 and 18.01 μmoles respectively in the liver. There was a significant decrease in the activity of the SDH, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase and ACP enzymes, whereas the activity of LDH, ASAT, ALAT, and AKP was increased significantly when compared with those of the corresponding parameters of the control mice.

In the female mice treated with 24 mg indoxacarb, the activity of LDH, SDH, ASAT, ALAT, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase, ACP and AKP enzymes was 15.73, 10.03, 18.54, 18.03, 2.49, 3.46, 1.80, 11.10 and 18.96 μmoles respectively in the liver. There was a significant decrease in the activity of SDH, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase and ACP enzymes, whereas the activity of LDH, ASAT, ALAT and AKP was increased significantly when compared with those of the corresponding parameters of the control mice.

The findings of the present study on the activity of the dehydrogenase, aminotransferase and phosphatase enzymes of the liver in female mice revealed that treatment with 18 and 24 mg indoxacarb showed significant decrease in the activity of SDH, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase and ACP enzymes, whereas LDH, ASAT, ALAT and AKP enzymes activity was
increased significantly in the liver. However, treatment with 6 and 12 mg indoxacarb showed no significant change in the activity of enzymes in the liver when compared with that of the control mice.

d) Effect on liver dehydrogenase (LDH and SDH), aminotransferase (ASAT and ALAT) and phosphatase (ATPases, ACP and AKP) enzymes activity in male mice after exposure to indoxacarb (Table 3.4; Graph 3.4)

In the control male mice the activity of LDH, SDH, ASAT, ALAT, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase, ACP and AKP enzymes was 13.26, 13.70, 16.18, 14.89, 4.24, 8.01, 3.40, 13.60 and 16.53 μmoles respectively in the liver. Treatment with 6 mg indoxacarb, the activity of LDH, SDH, ASAT, ALAT, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase, ACP and AKP enzymes was 14.01, 12.96, 16.63, 15.08, 4.10, 7.36, 2.93, 13.20 and 17.16 μmoles respectively in the liver. There was no significant change in the activity of the enzymes when compared with that of the control mice.

In the male mice treated with 12 mg indoxacarb, the activity of LDH, SDH, ASAT, ALAT, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase, ACP and AKP enzymes was 14.70, 12.33, 17.06, 15.99, 3.26, 6.76, 2.38, 12.35 and 17.86 μmoles respectively in the liver. There was no significant change in the activity of the enzymes when compared with that of the control mice.

In the male mice treated with 18 mg indoxacarb, the activity of LDH, SDH, ASAT, ALAT, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase, ACP and AKP enzymes was 15.63, 11.46, 17.99, 16.99, 2.70, 6.30, 1.91, 11.50 and 18.93 μmoles respectively in the liver. There was a significant decrease in the activity of SDH, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase and ACP enzymes and significant increase in the activity of LDH, ASAT, ALAT and AKP enzymes when compared with that of the control mice.

In the male mice treated with 24 mg indoxacarb, the activity of LDH, SDH, ASAT, ALAT, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase, ACP and AKP enzymes was 16.33, 10.50, 18.94, 18.01, 2.3, 5.58, 1.55, 10.85 and 19.73 μmoles respectively in the liver. There was a significant decrease in the activity of the SDH, Na^+-K^+ATPase, Mg^{++}ATPase, Ca^{++}ATPase and ACP enzymes, whereas the activity of LDH, ASAT, ALAT and AKP enzymes was increased significantly when compared with that of the control mice.
The findings of the present study on the activity of the dehydrogenase, aminotransferase and phosphatase enzymes of the liver in male mice revealed that, the mice treated with 18 and 24 mg indoxacarb showed significant decrease in the activity of SDH, Na-K\(^{+}\)ATPase, Mg\(^{2+}\)ATPase, Ca\(^{2+}\)ATPase and ACP enzymes and significant increase in LDH, ASAT, ALAT and AKP enzymes activity. Treatment with 6 and 12 mg indoxacarb showed no change in the activity of dehydrogenase, aminotransferase and phosphatase enzymes in the liver when compared with that of the control mice.

e) Antioxidants and oxidative stress byproducts of the liver in female mice after exposure to indoxacarb (Table 3.5; Graph 3.5)

In the control female mice the level of GSH, TBARS, protein carbonyl and ascorbic acid was 1.75, 14.00, 1.40, and 430 respectively in the liver. In the female mice treated with 6 mg indoxacarb, the level of GSH, TBARS, protein carbonyl and ascorbic acid was 1.45, 18.3, 1.48, and 400 \(\mu\)g respectively in the liver. There was no significant change in the level of GSH, TBARS, protein carbonyl and ascorbic acid contents when compared with that of the control mice.

In the female mice treated with 12 mg indoxacarb the level of GSH, TBARS, protein carbonyl and ascorbic acid was 1.38, 22.20, 1.59 and 375 respectively in the liver. There was no significant change in the level of GSH, TBARS, protein carbonyl and ascorbic acid contents when compared with that of the control mice.

In the female mice treated with 18 mg indoxacarb, the level of GSH, TBARS, protein carbonyl and ascorbic acid was 1.30, 25.6, 1.65, and 345 respectively in the liver. However, the level of GSH and ascorbic acid was decreased significantly, whereas the level of TBARS, protein carbonyl was increased significantly when compared with that of the control mice.

In the female mice treated with 24 mg indoxacarb, the level of GSH, TBARS, protein carbonyl and ascorbic acid was 1.20, 28.0, 1.78, and 320 respectively. There was a significant decrease in the level of GSH and ascorbic acid. However, the level of TBARS and protein carbonyl was increased significantly when compared with that of the control mice.

Results of the present study on antioxidant and oxidative stress byproducts of the liver in female mice revealed that the treatment with 6 and 12 mg indoxacarb showed no significant
change in the level of antioxidant and oxidative stress byproducts whereas, treatment with 18 and 24 mg indoxacarb caused significant decrease in the level of GSH and ascorbic acid and significant increase in the level of TBARS and protein carbonyl when compared with that of the control mice.

f) Oxidative stress enzymes (catalase [CAT], super oxide dismutase [SOD] and glutathione-s-transferase [GST]) of the liver in female mice after exposure to indoxacarb (Table 3.5; Graph 3.6)

In the female control mice, the activity of the catalase, SOD, and glutathione s-transferase enzymes was 0.045, 46.18 and 0.83 μmoles respectively in the liver. Treatment with 6 mg indoxacarb, the activity of the catalase, SOD, and glutathione s-transferase was 0.047, 47.08 and 0.88 μmoles respectively in the liver. 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 female mice treated with 12 mg indoxacarb the activity of glutathione s-transferase, SOD and catalase enzymes was 0.049, 47.90 and 0.95 μmoles respectively in the liver. There was no significant change in the activity of the enzymes, when compared with that of the control mice.

In the female mice treated with 18 mg indoxacarb, the activity of catalase, SOD, and glutathione s-transferase was 0.055, 48.55 and 1.03 μmoles respectively in the liver. There was a significant increase in the activity of the catalase, SOD, and glutathione s-transferase. When compared with that of the control mice.

In the female mice treated with 24 mg indoxacarb, the activity of catalase, SOD, and glutathione s-transferase enzymes was 0.059, 49.65 and 1.08 μmoles respectively in the liver. There was a significant increase in the activity of glutathione s-transferase, SOD and catalase enzymes, when compared with those of the corresponding parameters of the control mice.

The findings of the present study on the activity of the oxidative stress enzymes of the liver in female mice revealed that treatment with 18 and 24 mg indoxacarb showed significant increase in the activity of catalase, SOD, and glutathione s-transferase enzymes. However, treatment with 6 and 12 mg indoxacarb showed no significant change in the activity of enzymes in the liver when compared with those of the corresponding parameters of the control mice.
g) Antioxidants and oxidative stress byproducts of the liver in male mice after exposure to indoxacarb (Table 3.6; Graph 3.7)

In the control male mice the level of GSH, TBARS, protein carbonyl and ascorbic acid was 1.70, 16.70, 1.35, and 415 respectively in the liver. In the male mice treated with 6 mg indoxacarb, the level of GSH, TBARS, protein carbonyl and ascorbic acid was 1.59, 20.90, 1.40, and 400 respectively in the liver. There was no significant change in the level of GSH, TBARS, protein carbonyl and ascorbic acid contents when compared with that of the control mice.

In the male mice treated with 12 mg indoxacarb the level of GSH, TBARS, protein carbonyl and ascorbic acid was 1.50, 23.9, 1.53, and 388.00 µg respectively in the liver. There was no significant change in the level of GSH, TBARS, protein carbonyl and ascorbic acid contents when compared with those of the corresponding parameters of the control mice.

In the male mice treated with 18 mg indoxacarb, the level of GSH, TBARS, protein carbonyl and ascorbic acid was 1.35, 28.8, 1.59, and 350.00 respectively in the liver. There was a significant decrease in the level of GSH, ascorbic acid. However, the level of TBARS and protein carbonyl was increased significantly when compared with that of the control mice.

In the male mice treated with 24 mg indoxacarb, the level of GSH, TBARS, protein carbonyl and ascorbic acid 1.23, 32.50, 1.65, and 325 respectively in the liver. There was a significant decrease in the level of GSH and ascorbic acid contents. However, the level of TBARS and protein carbonyl was increased significantly when compared with that of the control mice.

Results of the present study on antioxidant and oxidative stress byproducts of the liver in male mice revealed that treatment with 18 and 24 mg indoxacarb caused significant decrease in the level of GSH, ascorbic acid and significant increase in the level of TBARS, protein carbonyl, whereas treatment with 6 and 12 mg indoxacarb caused no significant change in the levels of antioxidant and oxidative stress byproducts, when compared with that of the control mice.
h) Oxidative stress enzymes (catalase [CAT], super oxide dismutase [SOD] and glutathione s-transferase [GST]) of the liver in male mice after exposure to indoxacarb (Table 3.6; Graph 3.8)

In the control male mice the activity of catalase, SOD, and glutathione s-transferase enzymes was 0.042, 45.5, and 0.91 μmoles respectively in the liver. Treatment with 6 mg indoxacarb, the activity of catalase, SOD, and glutathione s-transferase enzymes was 0.045, 46.5 and 0.95 μmoles respectively in the liver. 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 male mice treated with 12 mg indoxacarb, the activity catalase, SOD, and glutathione s-transferase enzymes was 0.048, 46.90 and 1.05 μmoles respectively in the liver. 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 male mice treated with 18 mg indoxacarb, the activity of catalase, SOD, and glutathione s-transferase enzymes was 0.054, 47.50 and 1.20 μmoles respectively in the liver. There was a significant increase in the activity of catalase, SOD, and glutathione s-transferase enzymes when compared with those of the corresponding parameters of the control mice.

In the male mice treated with 24 mg indoxacarb, the activity of catalase, SOD, and glutathione s-transferase enzymes was 0.058, 48.25 and 1.25 μmoles respectively in the liver. There was a significant increase in the activity of the catalase, SOD, and glutathione s-transferase enzymes, when compared with those of the corresponding parameters of the control mice.

The findings of the present study on the activity of the oxidative stress enzymes of the liver in male mice revealed that, the mice treated with 18 and 24 mg indoxacarb showed significant increase in catalase, SOD, and glutathione s-transferase enzymes activity. Treatment with 6 and 12 mg indoxacarb showed no change in the activity of catalase, SOD, and glutathione-s-transferase enzymes in the liver when compared with those of the corresponding parameters of the control mice.
i) Effect on body and organs weight in female mice after exposure to indoxacarb (Table 3.7; Graph 3.9)

Body weight

In the control female mice change in body weight was 2.89 g when compared with that of the initial body weight. In mice treated with 6, 12, 18 and 24 mg indoxacarb, the relative change in body weight was 2.74, 2.62, 2.48 and 1.97 g respectively. There was significant decrease in the body weight gain in the mice treated with 18 and 24 mg indoxacarb. However, in the mice treated with 6 and 12 mg indoxacarb, caused no significant change in the body weight gain when compared with that of the control mice.

Organs weight

In the control female mice, the mean weight of the liver was 7.56 g. The mean weight of the liver with 6, 12, 18 and 24 mg indoxacarb was 7.12, 6.54, 6.03 and 5.33 g respectively. In the mice treated with 18 and 24 mg indoxacarb the liver weight was decreased significantly. However, mice treated with 6 and 12 mg indoxacarb the liver weight was not changed significantly when compared with that of control mice.

Liver histology

Histologic observations of the liver in control mouse revealed normal structure of the liver lobules. The hepatic lobules possess the rows of cuboidal or polyhedral hepatic cells forms hepatic cords from the center to periphery of the lobule. Hepatic cords arranged radially around the central vein. Between hepatic cords however narrow irregular spaces the lacunae through which hepatic sinusoids runs (Fig.1). In the mice treated with 6 and 12 mg/kg/day indoxacarb for 30 days, histologic study of the liver exhibited normal structure of liver lobules showing hepatic cords with cuboidal or polyhedral hepatic cells with sinusoids (Fig. 2&3). In the mice treated with 18 mg/kg/day indoxacarb for 30 days, histologic study of the liver revealed dilation of central vein and sinusoids between hypertrophied hepatocytes. Vacuolization and hyalinization of hepatocytes with loss radial arrangements (Fig. 4). In the mice treated with 24 mg/kg/day indoxacarb for 30 days, histologic observations revealed vacuolization, hypertrophy and hyalinization of hepatocytes with more dilation of central vein and radial arrangement of hepatocytes were lossed (Fig. 5).
Thyroid

In the control female mice, the mean weight of the thyroid was 4.32 mg. The mean weight of the thyroid with 6, 12, 18 and 24 mg indoxacarb treatment was 4.16, 3.89, 3.13 and 2.61 mg respectively. In the mice treated with 18 and 24 mg indoxacarb thyroid weight was decreased significantly. However, in the mice treated with 6 and 12 mg indoxacarb caused no significant change in thyroid weight when compared with that of control mice.

Thymus

In the control female mice, the mean weight of the thymus was 116.30 mg. The mean weight of the thymus with 6, 12, 18 and 24 mg indoxacarb treatment was 110.5, 95.72, 76.87 and 62.33 mg respectively. There was a significant decrease in the thymus weight with 18 and 24 mg indoxacarb. However, 6 and 12 mg indoxacarb showed no significant change in the thymus weight when compared with that of control mice.

Spleen

In the control female mice, the mean weight of the spleen was 419.76 mg. The mean weight of the spleen with 6, 12, 18 and 24 mg indoxacarb treatment was 398.79, 335.57, 288.72, 245.25 mg respectively. There was a significant decrease in the spleen weight with 18 and 24 mg indoxacarb. However, mice treated 6 and 12 mg indoxacarb caused no significant change in the spleen weight, when compared with that of control mice.

Adrenals

In the control female mice, the mean weight of adrenals was 46.58 mg. The mean weight of adrenals with 6, 12, 18 and 24 mg indoxacarb treatment was 42.32, 35.53, 30.89 and 21.58 mg respectively. There was significant decrease in the weight of the adrenals in the mice treated with 18 and 24 mg indoxacarb. However, 6 and 12 mg Indoxacarb showed no significant change in the adrenals weight when compared with that of the control mice.

The results of the present study on body and organs weight in female mice revealed that, the treatment with 18 and 24 mg indoxacarb caused significant decrease in the body weight gain and the organs thyroid, thymus, spleen, liver and adrenals. However, treatment with 6 and 12 mg indoxacarb caused no significant change in the body and organs weight when compared with those of the corresponding parameters of the control mice.
j) Effect on body and organs weight in male mice after exposure to indoxacarb (Table 3.8; Graph 3.10)

Body weight

In the control male mice change in body weight was 2.85 g when compared with that of the initial body weight. In the mice treated with 6, 12, 18 and 24 mg indoxacarb, the change in body weight was 2.75, 2.60, 2.40 and 1.90 g respectively. There was a significant decrease in the body weight gain, in the mice treated with 18 and 24 mg indoxacarb. However, 6 and 12 mg indoxacarb caused no significant change in the body weight gain when compared with that of the control mice.

Organs weight

In the control male mice, the mean weight of the liver was 7.46 g. The mean weight of the liver with 6, 12, 18 and 24 mg indoxacarb was 6.88, 6.37, 5.69 and 5.04 g respectively. There was a significant decrease in the weight of the liver in the mice treated with 12, 18 and 24 mg indoxacarb. However, 6 mg indoxacarb caused no significant change in the weight of the liver when compared with that of the control mice.

Thyroid

In the control male mice, the mean weight of thyroid was 4.38 mg. The mean weight of the thyroid with 6, 12, 18 and 24 mg indoxacarb was 4.11, 3.77, 3.24 and 2.48 mg respectively. There was no significant change in the thyroid weight with 6 and 12 mg indoxacarb. However, 18 and 24 mg indoxacarb showed significant decrease in the thyroid weight when compared with that of the control mice.

Thymus

In the control mice, the mean weight of the thymus was 122.40 mg. The mean weight of the thymus with 6, 12, 18 and 24 mg indoxacarb was 107.30, 89.36, 67.85 and 41.91 mg respectively. There was a significant decrease in the weight of the thymus in the mice treated with 18 and 24 mg indoxacarb. However, 6 and 12 mg indoxacarb caused no significant change in the thymus weight compared with that of the control mice.
Spleen

In the control male mice, the mean weight of the spleen was 422.10 mg. The mean weight of the spleen with 6, 12, 18 and 24 mg Indoxacarb was 400.43, 357.14, 322.64 and 300.94 mg respectively. There was a significant decrease in the weight of the spleen in the mice treated with 18 and 24 mg Indoxacarb. However, 6 and 12 mg Indoxacarb caused no significant change in the weight of the spleen when compared with that of the control mice.

Adrenals

In the control male mice, the mean weight of the adrenals was 45.93 mg. The mean weight of the adrenals with 6, 12, 18 and 24 mg Indoxacarb was 38.52, 35.16, 29.95 and 22.94 mg respectively. There was a significant decrease in the weight of the adrenals in the mice treated with 18 and 24 mg Indoxacarb. However, 24 and 18 mg indoxacarb caused no significant change in the weight of the adrenals when compared with that of control mice.

The results of the present study on body and organs weight in male mice revealed that, in the mice treated with 18 and 24 mg indoxacarb caused significant decrease in the body weight gain and organs thyroid, thymus, spleen, liver and adrenals weight. However, mice treated with 6 and 12 mg indoxacarb caused no significant change in the body and organs weight when compared with those of the corresponding parameters of the control mice.

k) Agarose gel electrophoresis of cellular DNA in the liver of mice after exposure to indoxacarb

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

a) Effect of indoxacarb on liver in mice

The basic structure of the liver consists of rows of hepatic cells (hepatocytes or parenchymal cells) perforated by specialized blood capillaries and sinusoids. The sinusoid walls contain phagocytic cells called Kupffer cells whose role is to engulf and destroy materials such as solid particles, bacteria, dead blood cells, and so on. The main blood supply comes to the liver from the intestinal vasculature. These vessels, along with those from the spleen and the stomach, merge with each other to form the portal vein. On entering the liver, the portal vein subdivides and drains into the sinusoids. The blood then perfuses the liver and exits by the hepatic veins, which merge into the inferior vena cava and return blood to the heart. The hepatic artery supplies the liver with oxygenated arterial blood.

In the liver three main functions occur: storage, metabolism, and biosynthesis. Glucose is converted to glycogen and stored; when needed for energy, it is converted back to glucose. Fat, fat-soluble vitamins and other nutrients are also stored in the liver. Fatty acids are metabolized and converted to lipids, which are then conjugated with proteins synthesized in the liver and released into the bloodstream as lipoproteins. The liver also synthesizes numerous functional proteins, such as enzymes and blood-coagulating factors. In addition the liver, contains numerous xenobiotic metabolizing enzymes, is the main site of xenobiotic metabolism.

The liver, the largest organ in the body, is often the target organ for chemically induced injuries. Several important factors are known to contribute to the liver's susceptibility. First, most xenobiotics enter the body through the gastrointestinal (GI) tract and, after absorption, are transported by the hepatic portal vein to the liver: thus the liver is the first organ perfused by chemicals that are absorbed in the gut. A second factor is the high concentration in the liver of xenobiotic metabolizing enzymes, primarily the cytochrome P450-dependent monooxygenase system. Although most biotransformation is detoxification reactions, many oxidative reactions produce reactive metabolites that can induce lesions within the liver. Often areas of damage are in the centrilobular region, and this localization has been attributed, in part, to the higher concentration of cytochrome P450 in that area of the liver. Other materials, such as bile acids and many xenobiotics, move from the hepatocytes into the bile-carrying canaliculi, which merge into
larger ducts that follow the portal vein branches. The ducts merge into the hepatic duct from which bile drains into the upper part of the small intestine, the duodenum. The gall bladder serves to hold bile until it is emptied into the intestine.

Cell necrosis is a degenerative process leading to cell death. Necrosis, usually an acute injury, may be localized and affect only a few hepatocytes (focal necrosis), or it may involve an entire lobe (massive necrosis). Cell death occurs along with rupture of the plasma membrane, and is preceded by a number of morphologic changes such as cytoplasmic edema, dilation of the endoplasmic reticulum, disaggregation of polysomes, accumulation of triglycerides, swelling of mitochondria with disruption of cristae, and dissolution of organelles and nucleus. Biochemical events that may lead to these changes include binding of reactive metabolites to proteins and unsaturated lipids (inducing lipid peroxidation and subsequent membrane destruction) disturbance of cellular Ca\(^{2+}\) homeostasis, interference with metabolic pathways, shifts in Na\(^+\) and K\(^+\) balance, and inhibition of protein synthesis. Changes in blood chemistry resemble those seen with fatty liver, except they are quantitatively larger. Because of the regenerating capability of the liver, necrotic lesions are not necessarily critical. Massive areas of necrosis, however, can lead to severe liver damage and failure.

Liver is an organ of immense importance due to its peculiar anatomy and metabolic functions which are exactly meant for efficient removal of the toxins. The liver serves many metabolic functions. Toxic liver injury can reproduce virtually any known pattern of injury, including necrosis, steatosis, fibrosis, cholestasis, and vascular injury (Ishak and Zimmerman, 1995). Attributing liver injury to a toxic reaction is therefore, difficult (Benichou, 1990; Maria and Victorino, 1997). Although many pharmaceuticals cause liver injury, most hepatotoxic drug reactions are idiosyncratic, due to immunologic mechanisms or variations in host metabolic response (Lee, 1995). Metabolism of xenobiotics to a large extent takes place in the liver. The by-products of such metabolism sometimes become more toxic than the initial substance.

In the present study, liver histologic observations of the control mouse showed radially arranged hepatic cords around the central vein. The hepatocytes with centrally located nuclei, the histologic study of liver of the mice treated with 6 and 12 mg showed dilated central vein with swollen hepatocytes with pyknotic nuclei, vacuoles and hyalinization, radial arrangement of hepatocytes lost. The histologic examination of the liver of the mice treated with 18 and 24
mg indoxacarb revealed that hepatocytes adjacent to the central vein are spared dilation of central vein and sinusoids between hypertrophied hepatocytes. Cytoplasmic vacuolization and hyalinization of hepatocytes with loss of radial arrangement.

It has reported that the oral dosing of a carbamate insecticide carbosulfan at a range of 100 to 400 mg for 6 weeks caused liver damage in chickens and the histopathologic features showed central veins, arterioles, capillaries and hepatic sinusoids were dilated, large focal areas of necrosis and hemorrhages in the liver parenchyma, increase in binucleated cells (Athani et al., 1997). It has been observed that, treatment with endosulfan (10 mg/kg/day) in rats caused liver damage that includes dilation of sinusoidal spaces with irregular nuclear shape, degenerative changes includes binucleated cells, hypertrophy of hepatocytes and lymphocytic infiltration in central vein (Choudhury et al., 2003). Rats treated with permethrin (620 mg/kg/day) and DDT (12 mg/kg/day) separately causes liver damage and the histopathologic study showed hepatocytes with pyknotic nuclei, acidophilic cytoplasm and cell with nuclear fragmentation induced by permethrin, whereas DDT causes cytoplasmic vacuolization and hepatocyte necrosis (Kostka et al., 2000). Acetaminophen, an antipyretic drug caused acute pathogenesis and liver failure which has been attributed to the accumulation of toxic metabolites in hepatocytes (Dablin et al., 1984; Hinson et al., 1995). Benomyl a fungicide induced marked histopathological alternations in the liver tissue of rats such as cytoplasmic vacuolization, leucocytic infiltration, congestion of blood vessels and fatty infiltration, in rats has been observed by Saber et al., (2004). Selmanoglu et al., (2001) have reported that rats treated with carbendazim caused histological changes in the liver such as, congestion, mononuclear cell infiltration, hydropic degeneration as enlargement of sinusoids and increase in the number of Kupffer cells. Male mice fed 5000 mg/kg carbendazim in diet showed centrilobular hypertrophy, necrosis and swelling in the liver (WHO/IPCS, 1993). Igedioh and Akinyele (1996) have observed necroticized cells and edema in the liver of rats given benomyl. Gray et al., (1990) reported that 400 mg/kg carbendazim did not cause any histopathological damage in the liver and kidney of rats. Prasanthi et al., (2005) have observed fenvelerate induced oxidative stress in hepatic tissue damage.

Shivanandappa and Krishnakumari (1981) have revealed the histopathologic changes in the liver of the rat treated with benzene hexachloride cyclohexane (BHC) an organochlorine insecticide, the hepatic histopathologic signs are hypertrophy, hyperplasia, vacuolization in
peripheral and centrilobular areas and focal necrotic areas also found and the histochemical observations revealed accumulation of cholesterol positive lipids in the periportal hepatocytes due to increase in total lipids content in the liver.

Methyl demeton, an organophosphate insecticide known to cause degenerative changes in hepatocytes includes necrosis and cytoplasmosolysis in rats (Dikshit et al., 1980a). In rabbits treatment with organophosphate insecticide phosphomidon alone and in combination with benzene causes hepatic changes. The dilation and congestion of sinusoids, ballooning of hepatocytes with pycnotic nuclei and focal necrosis was found (Dikshit et al., 1980b).

It has been reported that chloropyrifos, an organophosphorous pesticide caused oxidative stress and tissue damage in the liver and kidney, brain and fetus in pregnant rats (Zama et al., 2007). Sharma et al., (2005) have reported that dimethoate caused oxidative stress due to which inhibition of AChE and cytochrome P450 and disturbance in the activities of GSH and GST enzymes causing lipid peroxidation and histological changes in the liver and the brain.

In the present investigation with increase in dose and prolong oral administration of indoxacarb resulted in histopathological changes in the liver. The observed hepato histopathological changes are vacuolization and hyalization and loss of radical arrangement of hepatocytes, dilation of sinusoidal spaces with irregular nuclear shape (pyknotic nuclei) and increase in the number of Kupffer cells. From the observations it is thus indicated that increase in dose exposure of indoxacarb causes hepatic damages.

b) Effect of indoxacarb on biochemical contents (DNA, RNA, protein, glycogen and cholesterol) of the liver in mice

Nucleic acids

In the present graded dose exposure study, mice treated with 18 and 24 mg indoxacarb caused significant decrease in DNA and RNA of the liver in the mice. However, 6 and 12 mg indoxacarb treatment caused no significant change in DNA and RNA levels in the liver of mice. Similar findings have been reported that due to intoxication of carbosulfan decreases the nucleic acid contents in the liver of the (Ksheerasagar and Kaliwal, 2006). Similarly methomyl and
phosphomidon intoxication has reported to decrease the nucleic acid contents in the kidney and liver (Manawadi, 2006; Shreelakshmi, 2008).

It has been reported that by nuparion treatment the content of DNA in liver of male mice was significantly reduced and the reduction was 27.6%, 49% and 60% for the low, medium and high dose respectively. The same reduction was nearly obtained for liver-RNA content in both male and pregnant female mice by nuparion treatment. The reduction in DNA was also observed by several authors (El-Fiky et al., 1992; Hour et al., 1998; Blasik et al., 1999) who attributed this effect to the inhibition of DNA synthesis or DNA damage by the organophosphorous compounds. The insecticide was also found to be genotoxic and inducing severe DNA lesions (Aiub et al., 2002). The reduction in RNA contents was dependent on the decrease of total nucleic acids and total protein in liver and brain of animals using insecticides (El-Fiky et al., 1992), inhibition of its synthesis (Gale et al., 1971) or to the general inhibition of DNA dependent RNA polymerase (De Hondt et al., 1979). 2, 4-dichlorophenoxyacetic acid (2, 4-D) is a selective herbicide, hepatotoxic effects of 2,4-D have been well documented (Barenekow et al., 2000; Charles et al., 2001; Osaki et al., 2001). Several reports have demonstrated that 2,4-D can bind irreversibly to hepatic proteins in rat and chick (Di Paolo et al., 2001; Evangelista et al., 1993; Sulk et al., 1998). Li et al., (2001) indicated that 2,4-D-CoA may contribute to 2,4-D-protein adduct formation in vivo and therefore, associates hepatotoxicity. In mammalian cells in vitro, 2, 4-D inhibits cell growth, protein and DNA synthesis, and also arrests cells in the G/S phase of the cell cycle (Rivarol et al., 1985). Shivanandappa and Krishnakumari (1981) have reported that in the rats treated with BHC caused significant reduction in hepatic DNA and RNA, with an indication of cell death due to focal necrosis. Reduction of nucleic acids DNA and RNA was observed in the rats treated with benomyl in liver (Saber et al., 2004). Hellman and Laryea (1990) have reported that benomyl inhibited DNA turnover in the liver of mice by measuring the incorporation of [3H] thymidine 24 hrs after oral administration of different doses.

Abdel-Basset and Zaki (1990) observed reduction of RNA content in hepatocytes of rats intoxicated with fenvelerate. Similar reduction in RNA was recorded in animals treated with different pesticides. Dieldrin and Sevin were noted by Riad (1971) to induce RNA reduction in liver cells of Guinea pig. El-Ganzari (1975) observed that the insecticides lindane, quisathion and tamaron exerted an obvious reducing effect on RNA inclusions in rat liver cells. El-Elaimy and Husab El-Nabi (1990) reported that insecticide roger induced marked loss of RNA in liver cells.
of mice. Similarly the other carbamate pesticides such as benomyl and propoxur lead to formation of chromosomal breaks by breaking the phosphodiester backbone of DNA, and can induce aneuploidy and polyploidy by preventing the formation of spindles (Adhikari and Grover, 1988; Barale et al., 1993; Zelesco et al., 1990; Cid et al., 1990; Georgieva et al., 1990). The lipophilic characteristics of cypermethrin (CYP) indicate that the site of action is sodium channels in the neuronal membrane (Gabbianelli et al., 2002; Michelangeli et al., 1990; Narahashi, 1996) and it accumulates mostly in fat, skin, liver and kidney (WHO: 1989). Similar reports have been reported that due to intoxication of cypermethrin the concentrations of malondialdehyde (MDA) in the liver (63.3%), brain (31.8%) and kidney (21.1%) in alone CYP treated group were significantly higher than the control group. 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).

In the present study, the decreased levels of nucleic acids of the liver of mice are under the influence of indoxacarb treatment may be due to

a) The reduction in DNA attributed to the inhibition of DNA synthesis or DNA damage by the indoxacarb. The insecticide may be genotoxic and inducing severe DNA lesions (Aiub et al., 2002).

b) Formation of chromosomal breaks by breaking the phosphodiester backbone of DNA.

c) The reduction in RNA contents decrease of total nucleic acids and total protein in liver and brain of animals using insecticides (El-Fiky et al., 1992), inhibition of its synthesis (Gale et al., 1971) or to the general inhibition of DNA dependent RNA polymerase (De Hondt et al., 1979).

d) Production of reactive oxygen species (ROS)

Protein and glycogen

The results of the present graded doses of exposure study, treated with 18 and 24 mg indoxacarb caused significant decrease in the level of protein and glycogen in liver. However, 6 and 12 mg indoxacarb treatment showed no significant change in the level of protein and glycogen in liver. Similar reports have been reported that due to intoxication of carbofuran decrease the Protein and glycogen contents in liver (Ksheerasagar and Kaliwal, 2006). Similarly methomyl and phosphomidon intoxication has reported to decreases the Protein and glycogen contents in the kidney and liver (Manawadi, 2006; Shreelakshmi, 2008).

The obtained results agreed with other studies (El-Fiky et al., 1992; Prabhakaran et al., 1993) who found that organophosphorous pesticides decreased total serum proteins in the treated animals. They added that albumin (A) content was decreased while the globulins (G) were increased and therefore A/G was decreased (from 1.5 to 1.12). These changes were related to the physiological state and the health of animals hence the insecticide may affect the gastrointestinal tract and induce the decrease in the absorption and assimilation of protein (Zamanov, 1970; Hassan et al., 1989). The reduction in RNA contents was dependent on the decrease of total nucleic acids and total protein in liver and brain of animals using insecticides (El-Fiky et al., 1992), inhibition of its synthesis (Gale et al., 1971) or to the general inhibition of DNA dependent RNA polymerase (De Hondt et al., 1979). 2, 4-dichlorophenoxyacetic acid (2,4-D) is a selective herbicide, hepatotoxic effects of 2,4-D have been well documented (Barenkow et al., 2000; Charles et al., 2001; Osaki et al., 2001). 2, 4-D is capable of binding itself with proteins. On the one hand it can lead to detoxification of xenobiotic (conjugation with aminoacids and plasma albumins), and on the other it may cause essential disturbances in cell function and, finally, lead to its death (changes in enzymes activity) (Di Paolo et al., 2001). Protein damage may be the result of direct impact of 2,4-D or its indirect effect, for example by generation of free radicals (protein peroxidation). It has been suggested that there was a significant decrease in the microsomal protein Cyt-P450 content of the liver, lung, brain and kidneys of rats treated with pesticide vapacid (Mohd, 1993).

It has been observed an increase in the level of water-soluble proteins after 6 and 12 days of paraquat treatment and after 12-day exposure to methoxychlor, whereas 6-day injections of this pesticide did not significantly change protein content in comparison to the control group.
The protein content of tissues depends on the dynamic equilibrium between the rates of protein synthesis and protein degradation or redistribution between those soluble in water and those bound to cellular structures (Muhammad et al., 2003). Sancho et al., (1998) have reported a decrease in protein content of blood of fenitrothion intoxicated fish, and therefore they suggested that the decline in protein level indicates the physiological adaptability to compensate for pesticide stress in fish. To overcome the stress, they use more energy, which leads to stimulation of protein catabolism. Sancho et al., (1998) have also reported that the stimulation of protein synthesis results from the oxidative stress caused by the pesticides tested. Besides, it is possible that the increase in protein content can also be a consequence of dehydration of hepatocytes. Muhammad et al., (2003) have reported that the total protein contents of the liver in the frog (Rana tigrina) decreased after cypermethrin and permethrin intoxication (0.1% and 1% concentration at 1 ml dose) treatment. In the liver the reduction was 76% and 44% after cypermethrin treatment and 89% and 87% in permethrin-treated samples.

Rats treated with carbamate compound recorded a highly significant increase in serum glucose which may be due to increase glycogenolysis, decrease utilization of glucose by the tissue and/or increase gluconeogenesis, this agrees with the results on hens a decrease in liver glycogen level after administration of carbamate (Berberian and Enan, 1987; Anam and Metra, 1995) were recorded. The same data were observed by Dekundy et al., (2000) indicates an enhanced rate of glycolysis due to carbamate stress. It has been reported that lipophilic nature of carbamates may render them with the ability to interact with the serum and tissue lipids. Carbamates have been reported to cause alterations in the level of total serum lipids, glucose and protein levels in rats (Sadek et al., 1989).

In the present study the decrease in the level of proteins and glycogen in the liver of indoxacarb treated mice may be due to

a) Changes that were related to the physiological state and the health of animals hence the insecticide may affect the gastrointestinal tract and induce the decrease in the absorption and assimilation of protein (Zamanov, 1970; Hassan et al., 1989).

b) Pesticides those are capable of binding itself with proteins. On the one hand it can lead to detoxification of xenobiotic (conjugation with aminoacids and plasma albumins), and on
the other it may cause essential disturbances in cell function and, finally, lead to its death (changes in enzymes activity) (Di Paolo et al., 2001).

c) The decline in protein and glycogen level indicates the physiological adaptability to compensate for pesticide stress. To overcome the stress, they use more energy, which leads to stimulation of protein and glycogen catabolism.

**Cholesterol**

In the present graded dose exposure study, treatment with 18 and 24 mg indoxacarb caused significant increase in cholesterol level of the liver. However, treatment with 6 and 12 mg indoxacarb showed no significant change in cholesterol level of the liver in mice. Similar reports have been reported that due to intoxication of carbofuran increase the cholesterol contents in liver (Ksheerasagar and Kaliwal, 2006). Similarly methomyl and phosphomidon intoxication has reported to increase the cholesterol contents in kidney and liver (Manawadi, 2006; Shreelakshmi, 2008).

Hyperlipidemic effect has also been reported in rats due to treatment with an organophosphate pesticide, mirex. The elevation in serum total lipids and total cholesterol may be attributed to the stimulation of catecholamines, which stimulate lipolysis and due to the increase of fatty acid production (Fayez and Kilgore, 1992; Gupta et al., 1994; Zaahkouk et al., 1996; Dekundy et al., 2000). Recently it has been reported by Devendra et al., (2009) due to intoxication of carbofuran and cartap the elevation in total serum cholesterol level that was observed could be due to blockage of liver bile ducts causing reduction or cessation of its secretion to the duodenum subsequently causing cholestasis. The results from the previous investigation were also in the conformity with that reported by (Gupta et al., 1994) in the serum of carbofuran treated mice. The disruptions of formation of lipoprotein have been reported by Hassan et al., (1995) as one of the factors leading to accumulation of cholesterol in carbofuran treated mice. The aforesaid changes could be due to increased tissue lipogenesis via acceleration of acetyl CoA (Newsholme and Leech, 1985) to be the precursor of cholesterol biosynthesis. It has been also reported that due to intoxication of carbofuran resulted in the elevation of serum total cholesterol level (Samir et al., 2000).

Gupta et al., (1986) have reported that there was no elevated level of total lipids, cholesterol and phospholipids in liver, kidney and serum of Furadan-treated mice. It has been
reported that mancozeb and carbofuran treatments have altered the levels of protein, glycogen and total lipids in liver, uterus and ovary in intact and hemicastrated rats and mice (Mahadevaswami et al., 2000; Baligar and Kaliwal, 2001, 2002). Similar reports were also been observed with endosulfan and diamethoate treated mice (Hiremath, 2000; Mahadevaswami, 2002). The acute treatment with monocrotophos showed tissue specific inhibition of microsomal Cyt-P<sub>450</sub> in hepatic and extrahepatic tissues resulting in the loss of haemoprotein in rats (Siddiqui et al., 1987). Similar results were also reported in rats treated with dimethoate (Siddiqui et al., 1991). Diethyl dithiocarbamate inhibits hepatic cyt-P<sub>450</sub> dependent activity in rats (Stott et al., 1997). Maria et al., (2007) have reported that repeated dose of chloropyrifos administration caused alteration of P<sub>450</sub> mediated hepatic metabolism.

In the present study the increase in the cholesterol level of the liver in mice. May be due to

a) Inhibitory action of pesticide on Cyt-P<sub>450</sub> enzyme (Shivanandappa and Krishnakumari, 1981; Siddiqui et al., 1987; Stott et al., 1997; Maria Francesca Comet et al., 2007) or high affinity binding, thereby affecting the enzymes which are essential for cholesterol break up causing deposition of cholesterol in the tissue (Zarh et al., 2002).

b) The elevation in serum total lipids and total cholesterol may be attributed to the stimulation of catecholamines, which stimulate lipolysis and increase fatty acid production (Fayez and Kilgore, 1992; Gupta et al., 1994; Zaahkouk et al., 1996; Dekundy et al., 2000).

c) **Effect of indoxacarb on lactate dehydrogenase (LDH) and succinic dehydrogenase (SDH) activity in mice**

In the present graded dose exposure study, treatment with 18 and 24 mg indoxacarb caused significant increase in LDH, and decrease in SDH activity in the liver of mice. However, 6 and 12 mg indoxacarb treatment caused no significant change in LDH and SDH activity in the liver of mice. Similar reports were reported due to carbofuran intoxication in liver (Ksheerasagar and Kaliwal, 2006). Similarly methomyl and phosphomidon intoxication has reported to increase the LDH and decrease in the SDH activity in the kidney and liver (Manawadi, 2006; Shreelakshmi, 2008).

Increased permeability of cell and necrosis are usually characterized by rise in LDH activity (Radhaiah, 1985). The observed 7,12-Dimethylbenz (a) anthracene (DMBA) induced
increase in the activity of hepatic and renal LDH may be attributed to the enhanced enzyme synthesis (Smith et al., 1999; Spitsbergen et al., 2000; Miyata et al., 2001; Wijnhoven et al., 2001; Lindhe et al., 2002; Buters et al., 2003). Similar results have been reported with different animal species in response to heavy metals and pesticides (Natarajan, 1984; Sastry et al., 1988; Sastry and Shulka, 1994; Sharma and Gopal, 1995, Altuntas et al., 2002). Also, LDH level which indicate the energy demands are met by anaerobic respiration through increase in LDH activity. Moreover, several investigators have been reported that the oxygen consumption and the activities of liver respiratory enzymes (e.g. succinate dehydrogenase, malate dehydrogenase, NAD-isocitrate dehydrogenase) were decreased considerably with an elevation of glucose-6-phosphate dehydrogenase, glyceraldehyde dehydrogenase and/or LDH activities in stressed animals. It has been suggested that the stressed animals are meeting its energy requirements through anaerobic oxidation (Balavenkatasubbaiah et al. 1984, Prasada et al., 1985; Bahskaran, 1988; Rajeswari et al., 1989; Gerbracht et al., 1990; Sharma and Gupta, 1990; Reddy et al., 1994; James et al., 1996; Gupta et al., 1997; Vaglio and Landriscina, 1999; Das and Mukherjee, 2000). Moreover, Rady et al., (1980) have showed that the carcinogenic urethane, dimethylnitrosamine (DMNA), 3-methylcholanthrene (MCA), Benzo(a)pyrene (BP), DMBA and aflatoxin B1 enhanced the activities of glycolytic enzymes (hexokinase, phosphofructokinase, pyruvate kinase and lactate dehydrogenase) in mouse lung. Sharma (1999) has reported that significant decrease in the activity of liver succinate dehydrogenase suggests that anaerobic metabolism was favored over aerobic oxidation of glucose through Krebs cycle in order to mitigate the energy crisis for survival. It is also evident that lactate dehydrogenase activity exhibited a significant increase in case of carbamate treated group. It was also noticed that the use of both antioxidant ameliorate this effect Samir et al., (2000). LDH enzyme system plays a principal role in the glycolytic cycle in the cell for conservation of stored energy (i.e. pyruvate or lactate), this enzyme is released by injury to many different tissues (Hamdy, 1993; Lohitnavy and Sinhasan, 1998). The LDH release is commonly used as a marker for necrotic/ osmotic cell death (Lash et al., 1995; Valentovic and Ball, 1998). Endosulfan alters the activities of lactic dehydrogenase, glucose-6-phosphate dehydrogenase and alkaline phosphatase, and decreases mitochondrial energy production in mice (Tietz, 1999)

In the present study the increase in dose of indoxacarb showed increase in LDH and decrease in SDH activity in liver of mice may be due to

b) Effect of pesticide on carbohydrate metabolism in the tissue is indicated by decrease in SDH activity (Guraya, 1985; Preidkalns and Weber, 1968) as this enzyme is related with high metabolic activity such as absorption and secretion (Padykula, 1952).

d) **Effect of indoxacarb aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activity of the liver in mice**

Transaminases (ASAT and ALAT), represent a group of enzymes that are present within the cytoplasm of the living cells. The highest concentrations of ALAT are found in liver tissue; while lower concentrations exist in heart muscles and relatively small amounts present in brain, kidney and serum. ASAT was found to have its highest concentration in a variety of tissues including liver, kidney, brain, skeletal and cardiac muscles (Cook, 1974).

The results of the present findings revealed that, with increase in dose exposure to indoxacarb showed increase in ASAT and ALAT activity of the liver in mice. Similar reports were reported due to carbofuran intoxication in liver (Ksheerasagar and Kaliwal, (2006). Similarly methomyl and phosphomidon intoxication has reported the increase in the Transaminases (ASAT and ALAT) activity of kidney and liver in mice (Manawadi, 2006; Shreelakshmi, 2008).

It has been reported that the increase in the activity of ASAT and ALAT may be due to the hepatic potency of carbamate resulting in destructive changes in the hepatic cells. The carbamate was administered orally and, hence, it reaches the liver first through the hepatic portal vein. The effect of the carbamate on the liver is in accordance with Kiran *et al.*, (1988) who reported that carbamate stimulates ASAT and ALAT of the liver *in vivo* and *in vitro*. They added that the observed stimulation of ALAT activity is due to carbamate interaction with the enzyme.
The elevation in transaminases activity that was noticed in carbamate treated suggests that existence of heavy drain during carbamate stress, which is known to induce elevation of serum transaminases (Kulkarni and Mehrotra, 1973). From another point of view, elevations of transaminases activity in blood have been considered as indicator of tissue damage, without any specific damage of one organ. Damaged cells release transaminases into blood stream, and factors such as alteration in permeability of cell membrane, increased synthesis or decreased enzyme degradation may be involved. Possible mechanisms involved in the elevation of serum ALAT may be related to tissue damage (Korstad et al., 1972). Srivastava et al., (1989) have reported that ASAT and ALAT levels were increased significantly in plasma, liver, kidney, lung, brain, heart, intestine and muscle of rat treated with dichlorvos and suggested that these results might be due to cellular damage or increased permeability of plasma membrane. Similarly, increased levels of plasma ASAT and ALAT has also been reported in rats treated orally with monocrotophos (Janardhana and Sisod, 1990). Similarly, increased levels of plasma ASAT and ALAT has been reported in rats treated orally with dodine (Petrova et al., 1981), and serum ASAT in quinolphos treated Buffalo calves (Paul et al., 1984). Rahman et al., (2001) have reported increased levels of ASAT and ALAT activities in liver, kidney and lungs of rats treated with vepacide and suggested that these results might be due to increased tissue synthesis of these enzymes as an adaptive mechanism to the chemical stress. Both the transaminases (ASAT and ALAT) are important in protein metabolism. As for as the decrease in ASAT and ALAT activities in hepatocytes of DMBA-intoxicated Rana ridibunda, is considered it has also been observed that in different species linear alkalyibenzone sulphonate, pesticides, cadmium, lead and mercury intoxications strongly depressed ASAT and ALAT activities as a consequence of serious cellular structure damage (Vaglio and Landriscina, 1999; Gill et al., 1991a, b; Shakoori et al., 1994; Rahman and Siddiqui, 2003). In addition, the decrease in frog liver ALAT activity may be correlated with the fact that there is deficient conversion of alanine to Pyruvate which enters into Krebs cycle to compensate for energy requirement. ASAT is specific for glutamate and alpha-ketoglutarate but also reacts with nearly all amino acids. The depletion in the activities of ASAT and ALAT indicates disruption of link between carbohydrate and protein metabolism providing source of keto acids for Krebs cycle and gluconeogenesis (Gupta et al., 1989). The total protein content was reduced in the liver and gills of fish subjected to phenol. This may show that the protein was taken as an alternative source of energy, due to high energy demand that induced by phenol intoxication (Hori et al., 2006). This result is confirmed by the marked rise of ASAT and ALAT activities that reported in the present study. The enhanced activities of
transaminases induced tissue proteolysis. This phenomenon is previously recorded for different fish species subjected to phenol (Dange, 1986; Abdel-Hameid, 1994; Barse et al., 2006; Hori et al., 2006).

Increased level of ASAT and ALAT in liver is the result of treatment and indicative of toxic necrosis (Poli et al., 1987). In the present investigation marked increase in liver ALAT and ASAT under stress of indoxacarb has been observed. This elevation in above said parameters has been well supported by Srinivasan and Radhakrishnamurthy (1977), Srivastava et al., (1989), Rao and Banerji (1990), Rahman et al., (1996), Rahman et al., (2001) and Sahni and Saxena (2001) in albino rat after β and γ isomers of hexachlorohexane treatment, in rat after dichlorvos treatment, in rat after arochlor 1260 treatment, in rats after Azadirachta indica treatment, in albino rats after Azadirachta indica treatment in Mus musculus after diefethialone treatment respectively. The increase in transaminases activity in the liver is indicative of liver damage. ALAT and ASAT elevated in the serum of mancozeb-treated rats. This is in agreement with the result of Sarkar et al., (2005) who found that these enzymes increased in sera of mice administered with mancozeb. Kackar et al., (1999) also reported that oral administration of mancozeb to male rats induced changes in the activities of ALAT, ASAT, alkaline phosphatase, lactate dehydrogenase and acetylcholinesterase throughout the period of the study in a dose-dependent manner. Furthermore, Lavric et al. (1990) reported that the fungicide bithionol sulfoxide at high doses caused hepatotoxicity including an increase in serum ASAT.

The rise in ASAT and ALAT activities of the liver in mice in the present study may be due to the a) Cellular damage causing permeability of plasma membrane alterations (Srivastava et al., 1989; Ramazzalto and Carlin, 1978) and leakage of enzymes causing enhanced release of lysosomal enzymes (Choudhary et al., 2003; Srivastava et al., 1989)
b) Protein was taken as an alternative source of energy, demand that induced by pesticides intoxication (Gupta et al., 1989; Hori et al., 2006).

e) Effect of indoxacarb on ATPases activity of the liver in mice

In the present graded dose exposure study in female and male mice treated with 18 and 24 mg indoxacarb caused significant decrease in Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase activities of the liver in mice. However, the mice treated with 6 and 12 mg indoxacarb showed
no significant change in the activities of ATPases of the liver in mice. Similar reports are reported due to carbofuran intoxication in the liver (Ksheerasagar and Kaliwal, 2006) and Kidney (Ksheerasagar, 2005). Similarly methomyl and phosphomidon intoxication has reported a decrease in Na\(^+\)-K\(^+\)ATPase, Mg\(^++\)ATPase, Ca\(^++\)ATPase activities in the kidney and liver (Manawadi, 2006; Shreelakshmi, 2008).

Membrane bound Na\(^+\)-K\(^+\)ATPase is an important enzyme utilizing the energy by ATP hydrolysis for transport of several cations. Na\(^+\), K\(^+\) and Mg\(^++\)ATPases were more in the number and mitochondrial fractions, Ca\(^++\) dependent ATPases was found to be high in mitochondrial fractions alone. This may be due to the importance of these enzymes in different metabolic activities involving different subcellular organelles and membrane transport (Arunakaran et al., 1985). Mg\(^++\)ATPase is involved in ATP synthesis through oxidative phosphorylation in mitochondria (Boyer et al., 1977). Mitochondrial disorganization as reported during pesticidal toxicities (Pardini et al., 1980) might cause inhibition of Mg\(^++\)ATPase calcium ions play a major role as an important intra cellular messenger in many tissues particularly in central nervous system. It is 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).

It has been reported that the environmental xenobiotics specially pesticides are known to have a strong affinity for interaction with membrane lipids (Antunes-Maderia and Maderia, 1987). Cell membrane is believed to be the site of action of insecticides by altering structural and functional integrity of cell membrane and also affects Na\(^+\)-K\(^+\)ATPase and Mg\(^++\)ATPase (Shaw et al., 1995; Rauchova et al., 1995). Pesticides exert biologic effect on ATPase system by partitioning in the enzyme complex (Kinter et al., 1972). These enzymes are well known targets of organochlorine and organophosphorus pesticide compounds (Brown and Sharma, 1976; Pala et al., 1991). It has been found that mevinphos inhibited Na\(^+\)-K\(^+\)ATPase and Mg\(^++\)ATPase in chicken spinal cord (Brown and Sharma, 1976). DDT, malathion, ethyl and methyl parathion decreased renal total ATPase and Na\(^+\)-K\(^+\)ATPase in rats (Riedel and Christenson, 1979; Jarmillo-Jurez 1989; Dala et al., 1991).

The activities of Na\(^+\)-K\(^+\) and Mg\(^++\)-ATPase were also decreased in rats exposed to pyrethroids (Rao, 1984) and fenvalarate inhibited Na\(^+\)-K\(^+\) and Mg\(^++\)-ATPase in different tissues of fish cyprinus (Reddy et al., 1991). Na\(^+\)-K\(^+\) and Mg\(^++\)-ATPase were also inhibited by
lorazepam in human foetal and adult brain in vitro in a dose dependent manner (Saha et al., 1989). Similarly, Mehrotra et al., (1989) reported that the three cyclodine compounds aldrin, dieldrin and endrin significantly inhibited total and calmodulin stimulated Ca^{2+}-ATPase activity *in vitro* and *in vivo* in rat brain synaptosomes and heart sarcoplasmic reticulum, besides this both low and high affinity Ca^{2+}-ATPase in rat brain was reported by carbamate pesticide propoxur (Babu et al., 1990). Anjum and Siddiqui (1990) reported decreased Ca^{++} in fish *Tilapia mossambica* brain by monocrotophos, dimethoate and diazinon. Pala et al., (1991) have reported that malathion, methyl and ethyl parathion inhibited the calmodulin activity in a concentration dependent manner with malathion being less effective than ethyl and methyl parathion. Insecticides such as methyl parathion and parathion are reported to inhibit activities of ATPases (Basha and Nayeemunnisa, 1993; Blasiak, 1995). Archana et al., (2001) have reported that an organophosphate herbicide anilofos causes inhibition of total ATPase, Na^{+}-K^{+}-ATPase and Mg^{2+}-ATPase in RBCs, brain and liver of rats and suggested that, ATPase inhibition may cause neuronal/ cellular dysfunction by affecting ionic transport across cell membrane. Dhanya et al., (2003) have reported significant inhibition of membrane Na^{+}-K^{+}-ATPase in brain, liver and RBC of rat treated with di (2-ethylhexyl) phthalate (DEHP). Mg^{2+}-ATPase is reported to be inhibited by chlordecone in kidney and brain of rats (Bansal and Desaiah, 1985) and pyrethroids exposed to rats (Rao et al., 1984).

Thus, the results obtained from the present observation indicate that, the indoxacarb caused significant inhibition of cell membrane Na^{+}-K^{+}-ATPase, an important enzyme utilizing the energy from ATP hydrolysis for transport of several actions. In the present study, the pesticide indoxacarb might have affected cell membrane because of their strong affinity for interaction with membrane lipids (Antuner-Madera and Madera, 1987) causing inhibition of membrane bound ATPase enzymes activity by affecting enzyme complex (Kinter et al., 1972; Basha and Nayeemunnisa, 1993; Shao et al., 1995; Rauchova et al., 1995; Mishra et al., 1998).

f) Effect of indoxacarb on acid phosphatase (ACP) and alkaline phosphatase (AKP) activity of the liver in mice

In the present graded dose exposure study in mice treated with 18 and 24 mg indoxacarb caused significant decrease in ACP activity with significant increase in AKP activity in the liver. However, mice treated with 6 and 12 mg indoxacarb, the ACP and AKP activity in kidney of female and male mice were not changed significantly. Ksheerasagar and Kaliwal, (2006) have similar reports due to carbofuran intoxication in liver. Similarly methomyl and phosphomidon
intoxication has reported the decrease in ACP activity with increase in AKP activity of the
kidney and liver (Manawadi, 2006; Shreelakshmi, 2008).

Increase in serum and tissue ACP and AKP may also be due to hepato cellular necrosis or
cellular leakage that serves as a biomarker for chemicals induced injury. The gradual and
significant elevation in both ACP and AKP levels in different tissues like plasma, liver, kidney,
lung, brain, testis, intestine and muscles, of rat treated 60 mg/ kg of dichlorvos were observed
(Srivastava et al., 1989). Increase of ACP and AKP levels in all tissues and blood may be due to
damage of organs, especially liver, bones, small intestine and kidney (Zimmerman and Henry,
1969). In contrast Nagoha et al., (1989) reported that the serum ACP and AKP were elevated
whereas kidney and liver ACP and AKP were reduced in chloroquine treated rats.

The results of the present findings revealed that, increase in dose of exposure to
indoxacarb caused decrease in ACP and increase in AKP activity in liver of female and male
mice. The decrease in ACP activity by pesticides as in the present study probably indicates an
altered transport of phosphate (Engstrom, 1964) and inhibitory effect on the cell growth and
proliferation (Goldfisher et al., 1964). This may be due to the effect on absorptive or secretory
surface of the cell membrane causing cellular leakage. The elevated AKP activity in kidney
which is suggestive of an increase in tissue synthesis of these enzymes as an adaptive mechanism
to chemical stress (Murphy and Porter, 1966; Janardhana et al., 1987; Kacker et al., 1997;
Mishra et al., 1998; Bushuva et al., 1970)

Effect of indoxacarb on body and organs (liver, thyroid, thymus, spleen and adrenals)
weight in mice

The results of the present study on body and organs weight revealed that the treatment of
indoxacarb with 18 and 24 mg indoxacarb caused significant decrease in change in the body
weight and organs liver, thyroid, thymus, spleen, and adrenals weight. However, mice treated
with 6 and 12 mg indoxacarb showed no significant change in the body and organs weight.
Similar observations have been made with mancozeb, monocrotophos and dimethoate treated
rats and mice (Adilaxmamma et al., 1994; Ratnasooriya et al., 1995; Kacker et al., 1999;
Radhika and Kaliwal, 2002; Mahadevaswami and Kaliwal, 2002; Shreelakshmi and Kaliwal,
2007). Shivanandappa and Krishnakumari (1981) have reported that the liver, kidney, adrenals,
spleen, brain and testis weights decreased in rats treated with BHC. Selmanoglu et al., (2001)
reported no significant change in absolute organ and body weight gain, but there was a decrease in the liver/body weight ratio of the rats exposed to carbendazim at the highest dose.

In the present study, the decrease in the body and organs weight with increase in dose of exposure of indoxacarb may be due to suppression towards food and water intake. Although food and water intake has not been measured in this study, this may be one of the reasons for low weight gain. The results of the findings are in conformity with the earlier studies (Lu and Kennedy, 1986). Significant decrease in the relative weight of liver in the present study is in support of the findings reported earlier (Prazedziecki et al., 1969; Janardhan and Sisoda, 1990; Subramaniam et al., 1991; Ratnasoorya et al., 1995; Kacker et al., 1999; Radhika and Kaliwal, 2002; Shreelakshmi and Kaliwal, 2007). Thus, the significant decrease in the relative weight of organs and body weight in the present study may be due to indoxacarb induced pathophysiological and histological alterations or functional impairments in organs.

**h) Effect of indoxacarb on oxidative stress parameters of the liver in mice**

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

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

In addition to SODs and CAT, the glutathione S-transferases (GSTs) are important in the oxidative stress response. GSTs belong to a family of phase II enzymes that catalyze the conjugation of GSH into a wide variety of electrophilic compounds (Mannervik et al., 1985; Hayes and Pulford, 1995; Gong et al., 2005). As a water-soluble tripeptide, glutathione is the most abundant intracellular small thiol molecule and a predominant defense against ROS in tissues. GSH reacts directly with ROS and electrophilic metabolites, protects essential thiol groups from oxidation, promotes the regeneration of a-tocopherol, and serves as a substrate for GSH-related enzymes, e.g. glutathione peroxidase (GPx) and glutathione s-transferases (Townsend et al. 2003). GSH plays an important role in the maintenance of the intracellular redox state and in the cellular defense against oxidative damage. Our finding that the level of GSH is decreased in the liver of mice is consistent with previous reports (Kalaiselvi and
Panneerselvam, 1998; Mosoni et al., 2004). The observed decrease in glutathione concentration in the liver of mice may be caused by the excessive oxidative damage due to free radicals.

In transgenic mice the rapid depletion of GSH in response to paraquat exposure has been reported. This GSH depletion may result from participation of GSTs in the removal and reduction of (hydro) peroxides at the expense of GSH utilization. The heightened paraquat sensitivity is also paradoxical in light of the general increase in GST expression in VP-hPXR mice. Paraquat is a quaternary nitrogen herbicide that causes toxic effects mainly via oxidative stress-induced mechanisms (Suntres et al., 2002). Because GSTs play an important role in the detoxification of products from oxidative stress, a protective effect was expected for VP-hPXR mice in which GST expression and activity were highly induced. Glutathione is an endogenous thiol antioxidant that has a multifaceted role in xenobiotic metabolism and is a first line of defense against oxidant-mediated cell injury (Sies et al., 1999). The level of GSH showed a drastic reduction (76%) after acute exposure. On the contrary, chronic carbofuran exposure resulted in significant increase (67%) in GSH levels as compared with control. A rapid and drastic reduction in GSH level has also been observed by (Cereser et al., 2001) after carbamate exposure.

It is clear from both acute and chronic carbofuran exposure results in perturbations in oxidative stress several studies provide evidence that antioxidants may be used as biomarkers of exposure to environmental pollutants (Di Giulio et al., 1993; Hasspiele et al., 1994; Regoli and Principato, 1998). Moreover, many report that various pesticides can induce oxidative stress in different tissues (Bagchi et al., 1995; Bachowski et al., 1998; Hassoun and Stohs, 1996). Endosulfan, an organochlorine pesticide, recently was identified in vitro as a chemical that reduced cortisol secretion by teleost adrenocortical cells, affected the activity of enzymes involved in oxidative stress, and increased levels of lipid hydroperoxides (Dorval et al., 2003; Dorval and Hontela, 2003). It has been reported that both the exclusive exposure of animals to dimethoate as well as combined intoxication with dimethoate and pyrantel tartrate triggered a decrease in the GSH content in rat liver only in the first period after intoxication (up to the 24th h). However, a greater decrease was observed after mixed intoxication. Corresponding results were obtained earlier by Wysocki and Zasadowski, (2005) who used a concentrate of technical dimethoate and pyrantel embonate. They observed a decrease in the concentration of hepatic GSH in the first period of the study; however, that decline lasted longer (up to 12 h) after
exclusive administration of dimethoate. These results are consistent with the findings of other authors referring to administration of various pesticides (Łukaszewicz-Hussain and Moniuszko-Jakoniuk, 2003; Sharma et al., 2005) administered various doses of dimethoate (0.6, 6 and 30 mg/kg b.w.) to rats for 30 days, and found a significant decline in the GSH content both in the liver and brain of the animals after doses of 6 and 30 mg/kg. Subchronic exposure to dimethoate caused a decrease in the GSH level and, simultaneously, an enhanced activity of GST and glutathione reductase (GR) in the liver and brain of the animals. It has been revealed that 8-week oral administration of chlorpyrifos (at a dose of 13.5 mg/kg b.w.) to rats (Goel et al., 2005) observed a decreased level of hepatic GSH. They suggested that under oxidative stress, the content of GSH used by glutathione-dependent enzymes decreases. Other authors Ahmed et al., (2000) imply that, apart from the above-mentioned mechanism of action, the reduced level of GSH may also be caused by its direct participation in free radical reactions initiated by organophosphate compounds (malathion). GSH may act independently by evoking detoxification of active forms of oxygen, e.g. H₂O₂, or reduction of lipid peroxidation. Sivapiriya et al., (2006) demonstrated that the administration of dimethoate and ethanol to mice induced lipid peroxidation and reduced the GSH pool. Depletion of GSH stores in the liver supports the hypothesis that reactive oxygen species generated during dimethoate and ethanol metabolism lead to oxidation of GSH and lipid peroxidation, and are responsible for their complex toxic effect. A decrease of hepatic GSH and GST under acute intoxication with dimethoate and ethanol may initially lead to losses of both of those variables from the liver to plasma. Moreover, GSH - a direct scavenger of free radicals - constitutes a substrate for GPx and GST. In their study, the activity of these enzymes was also reduced. Thus, this can be ascribed to the unavailability of reduced glutathione (Spodniewska and Zasadowski, 2008).

There have been studies showing that the activity of superoxide dismutase and glutathione peroxidase decreases significantly following exposure to 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin (TCDD) (al-Bayati et al., 1988; Hermansky et al., 1988). These phenomena were observed at a high dose of TCDD however, the inhibition of these enzymes may only have been responsible for the acute toxic responses. Lipid peroxidation (LPO), the oxidative catabolism of polyunsaturated fatty acids, is widely accepted as a general mechanism for cellular injury and death (Gutteridge et al., 1983; Halliwell et al., 1984). LPO and free radical generation are complex and deleterious processes that are closely related to toxicity (Murray et al., 1988) LPO has been implicated in diverse pathological conditions, including atherosclerosis (Holvoet and
Collen, 1998), aging (Spiteller et al., 2001), rheumatoid arthritis (Henrotin et al., 1992), and cancer (Marnett et al., 2000). It is also involved in the toxicity of pesticides (Bismuth et al., 1990), solvents (Brattin et al., 1985) and metals (Kasprzak et al., 1995). The extension of the oxidative catabolism of lipid membranes can be evaluated by several endpoints, but the most widely used method is the quantification of malondialdehyde (MDA), one of the stable aldehydic products of lipoperoxidation, present in biological samples (De Zwart et al., 1999; Gutteridge et al., 1995).

Lipid peroxidation or an increase in the oxidized (GSSG) glutathione/reduced (GSH) glutathione ratio, which is a marker of oxidative stress (Stadtman and Berlett, 1994), was also observed when DNA damage occurred (Stohs et al., 1990; Tritscher et al., 1996; Park et al., 1996), and increased $O_2^-$ production has been detected in peritoneal lavage cells of SD rats) and peritoneal exudate cells of C57BL/6J and DBA/2 mice). It is well known that reactive oxygen species (ROS), such as the OH radical (·OH) and $O_2^-$, induce DNA breaks, 8-oxodG, lipid peroxidation (Vallyathan et al., 1998), and increase of the GSSG / GSH ratio (Stadtman and Berlett, 1997). A does-response relation was observed in some of these studies (Wahba, 1989; Alsharif, 1994), and the dose regimens are considering all of these results as a whole, while TCDD did not induce DNA damage in most genotoxicity tests, it did induce oxidative DNA damage or increase oxidative stress in several situations, which shows that TCDD is able to induce ROS, even if indirectly (Sahoo et al., 2000; Junqueira et al., 1997; Samanta et al., 1999; Abdollahi et al., 2004; Faraone-Mennella et al., 2003; Simon-Giavarotti et al.). Carbofuran has been reported to have high mammalian toxicity, and the main target organs are brain, liver, skeletal muscles, and heart (Gupta et al., 1994). Previously reported that chronic carbofuran exposure is responsible for oxidative injury leading to perturbations in membrane structure and functions (Kamboj et al., 2006). The levels of lipid peroxidation measured in terms of TBARS were observed to accentuate after both acute and chronic carbofuran exposure. The increase in lipid peroxidation was more (60%) in chronic carbofuran-exposed animals than acute exposure (14%). Milotovic et al., (2005) have also observed increase in lipid peroxidation in skeletal muscles after carbofuran exposure and have attributed this increase to increased formation of reactive oxygen and nitrogen species.

Several studies with liver, brain, and testes indicate that lindane causes oxidative stress (Sahoo et al., 2000; Junqueira et al., 1997; Samanta et al., 1999; Abdollahi et al., 2004; Faraone-Mennella et al., 2003; Simon-Giavarotti et al.).
Mennella, et al., 2003; Simon-Giavarotti et al.,). Increased myocardial TBARS and depletion of GSH provide evidence for oxidative stress. The increase in myocardial SOD and catalase activities might be due to adaptive changes following oxidative stress, as it has been reported earlier that myocardial adaptation following exposure to oxidative stress occurs through induction of antioxidant compounds (Das et al., 1995). A number of previous studies have reported that lindane causes oxidative stress in the liver (Khessiba, 2005; Videla, 1990; Junqueira, 1988).

Lipid peroxidation has been shown to increase in plasma and some tissues in Cypermethrin (CYP) and other insecticides toxicities (Parker et al., 1984; Akhtar et al., 1994; Gupta et al., 1999; Aldana et al., 2001). The lipophilic characteristics of CYP indicate that the site of action is sodium channels in the neuronal membrane (Gabbianelli et al., 2002; Michelangeli et al., 1990; Narahashi, 1996) and it accumulates mostly in fat, skin, liver and kidney (WHO: 1989), it has been reported that, the concentrations of MDA in the liver (63.3%), brain (31.8%) and kidney (21.1%) in alone CYP treated group were significantly higher than the control group. The increase in the concentrations of MDA is the indicator of CYP-induced lipid peroxidation. Studies indicate that pesticide intoxication produce oxidative stress by the generation of free radicals and induce tissue lipid peroxidation in mammals and other organisms (Çomelekoglu et al., 2000). Hincal et al., (1995) reported the oxidant stress inducing effects of endosulfan, with an increase of lipid peroxidation and a significant alteration in glutathione redox cycle in cerebral and hepatic tissues of rats. Furthermore, many of the metabolites of lindane are also conjugated with glutathione, causing depletion of the glutathione reserve (Kakker et al., 1984). This might be the mechanism underlying the decrease in glutathione levels in our study (Roy Ananya et al., 2005).

In the present study, the decreased levels of GSH and increased level of TBARS in the liver of mice under the influence of indoxacarb treatment may be due to

a) The indoxacarb is a fluorinated compound prone to bind various antioxidants and antioxidation enzymes as it has been observed oxidative stress in the liver 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 mice treated with 18 and 24 mg indoxacarb caused decrease in ascorbic acid and increase in the protein carbonyl of the liver in mice. However, 6 and 12 mg indoxacarb treatments caused no change in ascorbic acid and Protein carbonyl levels in the liver of mice.

Pyrantel tartrate administered to rats twice at the dose of 85 mg/kg b.w. did not cause any significant changes in the content of ascorbic acid in the liver. Throughout the experimental period, vitamin C concentration oscillated around control values. In an earlier study by Spodniewska and Zasadowski (2006), pyrantel embonate administered to rats at a dose of 1/5 LD50 for 3 consecutive days, was demonstrated to decrease the concentration of ascorbic acid in the final period of the experiment, i.e. on day 7 and 14, whereas the administration of pyrante1 embonate at the dose of 1/2 LD50 on day 14 and 28 of the experiment was observed to increase the vitamin C content in the liver 3 h after exposure. vitamin C-mediated quenching of mitochondrial ROS during normal and oxidative conditions correlate with the protective effect of vitamin C in inhibiting oxidative insults on the mitochondrial (mt DNA). Furthermore, since levels of mitochondrial ascorbic acid can be augmented with dietary vitamin C supplementation, our data suggest the pharmacological relevance of vitamin C in the protection of the mitochondrial genome against oxidative injury (Spodniewska and Zasadowski, 2006).

Once the rats were intoxicated with dimethoate in the form of a Bi 58 Nowy preparation, a decrease in vitamin C concentration was observed till the 3rd day of the experiment (inclusive). A similar tendency was observed after mixed intoxication with Bi 58 Nowy and pyrantel tartrate; however, the changes were less intensive. The diminished concentration of vitamin C may indicate intensification of oxidative stress, generation of free radicals, and damage to the cellular
membrane of hepatocytes as affected by the compounds applied in the experiment. A decreased vitamin C concentration in the liver was previously reported in a study by Spodniewska and Zasadowski (2006) after intoxication of rats with dimethoate (a technical concentrate) at a dose of 1/10 LD50. However, the decrease occurred from the 12th hour till the 7th day, which was not observed after 28-day intoxication with a dose of 1/25 LD50. Łukaszewicz-Hussain and Moniuszko-Jakoniuk (2003) have observed that when administering various doses of chlorfenvinphos to rats (0.02, 0.1 and 0.5 LD50), resulted in decrease in vitamin C level in serum, however, the decrease appeared to be greater after administration of lower doses of the pesticide. A reduced level of vitamin C in the testicles was also reported by Narayana et al., (2005) after intoxication of rats with methylparathion. Studies by Ikeda et al., (2003) demonstrated that paraquat administered to rats intraperitoneally at a dose of 50 mg/kg b.w. significantly decreased the level of ascorbic acid in the lungs, but not in the serum, liver or kidneys.

The observed decrease in vitamin C concentration in the liver may be also explained by its utilization for the regeneration of alpha-tocopherol, one of the elements of non-enzymatic antioxidant defense, whose content decreases under conditions of oxidative stress, which has been suggested by (Meister et al., 1992 ; Ficek et al., 1997). The decrease in the ascorbic acid content could also be due to the direct impact of the compounds examined (dimethoate and pyrantel) on the synthesis of vitamin C and metabolic pathways that require its presence. Similar suggestions were also postulated by Padh et al., (1991) and Sauberlich et al., (1994) who described the biochemical functions of vitamin C in the organisms.

Many environmental pollutants or chemicals exert their toxic effects by generating ROS (Klaunig and Kamendulis, 2004; Mori et al., 2007) ROS are unstable free radical species in cells produced when oxidative stress occurs (Klaunig and Kamendulis, 2004). These unstable free radical species can attack cellular components, inducing damage to lipids, proteins, and DNA and are associated with many disease states, including cancer (Mori et al, 2007). Proteins are major targets for ROS and can scavenge 50-75% of ROS, as they are the major component of most biological systems (Mori et al., 2007). Some ROS-induced protein modifications can result in unfolding or alteration of protein structure and functions (Karimpour et al., 2002) Protein targets of ROS are of increasing interest in environmental toxicity as they may provide insights to toxicity mechanisms and may identify novel biomarkers. ROS can modify and inactivate
proteins in a variety of ways (Fagan et al., 1999; Choy et al., 2008). Generally, ROS may cause reversible and/or irreversible modifications on sensitive proteins (Fagan et al., 1999). Reversible modifications, usually at cysteine residues, may have a dual role of protection from irreversible damage and modulation of protein function (Choy et al., 2008). Irreversible modifications induced by ROS such as carbonyl formation are generally associated with permanent loss of protein function, and are considered a widespread indicator of severe oxidative damage and disease-derived protein dysfunction (Fagan et al., 1999). Carbonyl groups are introduced into proteins by a variety of oxidative pathways (Fagan et al., 1999) as it has been observed in the present study. ROS can react directly with the protein by oxidizing amino acid residue side-chains into ketone or aldehyde derivatives or they can react with molecules such as sugars and lipids, generating reactive carbonyl species that then can react with protein. Although the overall biology of oxidative protein modifications remains complex and ill-defined, protein carbonylation is quite well characterized. Measurement of these carbonyl groups is thought to be a good estimation for the extent of oxidative damage of proteins associated with various conditions of oxidative stress, toxic process, and disease. Under oxidative stress, carbohydrates and lipids as well as proteins are the major targets of reactive oxygen species. Proteins are modified indirectly with reactive carbonyl compounds derived from the autoxidation of carbohydrates and lipids. Autoxidation of carbohydrates and ascorbate yields carbonyl compounds, e.g. glyoxal, arabinose, methylglyoxal, glycolaldehyde, and dehydroascorbate (Wells-Knecht et al., 1995; Ahmed et al., 1997; Glomb and Monnier, 1995; Miyata et al., 1997).

In mouse liver treated with propiconazole, and to probe possible mechanisms through which oxidative stress might contribute to the hepatic toxicity induced by propiconazole. Allen et al., (2006) has been reported that the percentage of liver weight/body weight and activity of liver toxicity marker GST were increased significantly in the liver of propiconazole-treated mice. Zarn, et al., (2003) have reported that protein carbonylation, but not thiol modification, in the mouse liver increased significantly during propiconazole induced oxidative stress. (INCHEM Part II Toxicology 1987) The GSH/GSSG ratio was reduced in the livers of propiconazole-treated mice, and the increased protein carbonylation could be a result of decreased GSH content.

Oxygen free radicals produced by the metal catalyzed oxidation systems have been assumed as the primary mechanism for the formation of protein carbonyls in vivo (Stadtman et al., 1992; Stadtman and Oliver, 1991) Protein carbonyls are also generated by oxygen free
radicals produced by ionizing radiation (Garrison et al, 1962.). The present conclusion that both glycoxidation and lipoxidation reactions contribute to the increase in protein carbonyls, is in good agreement with the assumption by (Stadtman, et al., 1992) that glycation or Maillard reaction may contribute to the formation of protein carbonyls.

In the present study, the decreased level of ascorbic acid and increased level of protein carbonyl in the liver of mice are under the influence of indoxacarb treatment which may be due to

a) Pollutants or chemicals exert their toxic effects by generating ROS (Klaunig and Kamendulis, 2004; Mori et al., 2007). ROS are unstable free radical species in cells produced when oxidative stress occurs (Klaunig and Kamendulis, 2004). Proteins are major targets for ROS and can scavenge 50-75% of ROS, as they are the major component of most of the biological systems (Mori et al., 2007). Some ROS-induced protein modifications can result in unfolding or alteration of protein structure and functions (Karimpour et al., 2002) Protein targets of ROS are of increasing interest in environmental toxicity as they may provide insights to toxicity mechanisms and may identify novel biomarkers.

b) The present conclusion that both glycoxidation and lipoxidation reactions contribute to the increase in protein carbonyls, is in good agreement with the assumption by (Stadtman et al., 1992) that glycation or Maillard reaction may contribute to the formation of protein carbonyls.

c) The diminished concentration of vitamin C may indicate intensification of oxidative stress, generation of free radicals, and damage to the cellular membrane of hepatocytes as affected by the compounds. A decreased vitamin C concentration in the liver was reported by (Spodniewska and Zasadowski, 2006) after intoxication of rats with dimethoate (a technical concentrate) at a dose of 1/10 LD50. The observed decrease in vitamin C concentration in the liver may be also explained by its utilization for the regeneration of alpha-tocopherol, one of the elements of non-enzymatic antioxidant defence, whose content decreases under conditions of oxidative stress, which has been suggested by (Meister et al., 1992; Ficek et al., 1997).
Super oxide dismutase (SOD) and catalase (CAT)

In the present graded dose exposure study the mice treated with 18 and 24 mg indoxacarb caused increase in the catalase, SOD and enzymes activity in the liver of mice. However, 6 and 12 mg indoxacarb treatments caused no change in catalase, SOD enzyme activity of levels in the liver of mice.

Enzymatic and non-enzymatic antioxidant protection (Yu, 1994), Glutathione peroxidase (GPx) and SOD are the main enzymes of the antioxidant defense system. A significant decline in the level of these enzymes in the liver of the mice observed in our study is in accordance with extensive studies (Kalaiselvi and Panneerselvam, 1998, Bejma et al., 2000, Kumaran et al., 2004; Savitha et al., 2005). GPx is a selenium-containing antioxidant enzyme, which effectively reduces hydrogen peroxide and lipid peroxides to water and lipid alcohols, respectively, at the expense of reduced glutathione.

The more efficient clearance of ROS, however, requires the coordinate actions of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT) (Rojkind, et al 2002). SOD catalyzes the conversion of O$_2$ - to H$_2$O$_2$ and O$_2$. In eukaryotic cells, there are two Cu/Zn-dependent SODs (SOD-1 and SOD-3) and one Mn-dependent SOD (SOD.$\alpha$). H$_2$O$_2$ is subsequently converted to water and O$_2$ by CAT, a tetrameric heme-containing enzyme complex (Rojkind et al 2002, Forsberg et al 2001). In vitro study also identified CAT, Gpx, and GSH as essential antioxidants in maintaining the function and integrity of rainbow trout (Oncorhynchus mykiss) adrenocortical cells and suggested that the glutathione redox cycle is more efficient than CAT in protecting adrenocortical cells against oxidative stress induced by endosulfan ( Dorval and Hontela, 2003). Lindane-induced oxidative stress in the heart, higher levels of SOD and catalase following adaptation might have protected the myocardium from more severe injury due to oxidative stress (Roy et al., 2005)

Oral administration of endosulfan caused oxidative stress in rat heart. As is reported by other authors, the activities of GPx are linked in their capacity to prevent peroxidative tissue damage from oxidants. GPx converts toxic lipid hydroperoxides and using reducing equivalents generated by G6PDH (Frei et al., 1989). However, in this study the increased activity of CAT,
SOD and GPx was observed at the same time after intoxication with endosulfan. Investigations indicated that mammalians have a good defense mechanism for lipid peroxidation because it can increase the hepatic CAT activity when needed. However, CAT is generally localized in peroxisomes and therefore, its role in the other parts of the cell is limited. In particular, \( \text{H}_2\text{O}_2 \) at low concentration is destroyed by this enzyme (Kale et al., 1999; Adal et al., 1999; Ner et al., 2001).

In the present study, the increased level of enzyme activities of catalase and superoxide dismutase increased in the liver of mice are under the influence of indoxacarb treatment may be due to the more efficient clearance of ROS, however, requires the coordinate actions of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT) (Rojkind et al., 2002). Investigations indicated that mammalians have a good defense mechanism for lipid peroxidation because it can increase the hepatic CAT activity when needed. However, CAT is generally localized in peroxisomes and therefore, its role in the other parts of the cell is limited as it has been observed in the present findings. In particular, \( \text{H}_2\text{O}_2 \) at low concentration is destroyed by this enzyme (Kale et al., 1999; Adal et al., 1999; Ner et al., 2001).

**Glutathione s-transferase (GST)**

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

In addition to SODs and CAT, the glutathione s-transferases (GSTs) are important in the oxidative stress response. GSTs belong to a family of phase II enzymes that catalyze the conjugation of GSH into a wide variety of electrophilic compounds (Mannervik et al., 1985; Hayes and Pulford, 1995; Gong et al., 2005). The heightened paraquat sensitivity is also paradoxical in light of the general increase in GST expression in VP-hPXR mice. Paraquat is a quaternary nitrogen herbicide that causes toxic effects mainly via oxidative stress-induced mechanisms (Suntres et al., 2002). Because GSTs play an important role in the detoxification of products from oxidative stress, a protective effect was expected for VP-hPXR mice in which GST expression and activity were highly induced. The sensitization in transgenic mice may be explained by the rapid depletion of GSH in response to paraquat exposure. This GSH depletion
may result from participation of GSTs in the removal and reduction of (hydro) peroxides at the expense of GSH utilization.

Glutathione-s-transferases are a major family of detoxifying enzymes that catalyze the conjugation of GSH with electrophilic centers of lipophilic substrates, thereby increasing its solubility and aiding their excretion from body depicts the activity of GST in liver of acute and chronic carbofuran-exposed animals (Vontas et al., 2001). A pronounced increase (131%) in the activity of GST was observed in animals chronically exposed to carbofuran. In the animals exposed to acute dose of carbofuran, the increase in GST activity was 24% of that seen in controls. Carbofuran has been reported to be metabolized in liver and is excreted as a conjugate of GSH by the reaction catalyzed by GST (Nigg and Knaak, 2001).

In mammals, GST greatly enhances its own activity by sulfhydryl-reactive metabolites and reduced oxygen species during oxidative stress (Moorhouse and Casida, 1992.) Increase in the activity of GST has been used as a marker of exposure to organochlorine contaminants (Machala et al., 1998). In the present study, GST activity may not differ between sites because GSH levels, essential to GST activity, are depleted at contaminated sites. Indeed, GST participates in pollutant detoxification by adding a GSH-group to xenobiotics or their metabolites, so they become more waters soluble and, thus, excreted more easily (Moorhouse and Casida, 1992). Mouse treated with propiconazole, and to probe possible mechanisms through which oxidative stress might contribute to the hepatic toxicity induced by propiconazole. The main findings of this study are: (Allen et al., 2006) the percentage of LW/BW and activity in liver toxicity marker GST were increased significantly in the liver of propiconazole-treated mice. (Zarn et al., 2003) Protein carbonylation, but not thiol modification, in the mouse liver increased significantly during propiconazole induced oxidative stress. (INCHEM Monograph 768 Propiconazole (Pesticide residues in food: 1987 Evaluations, Part II Toxicology 1987)) The GSH/GSSG ratio was reduced in the livers of propiconazole-treated mice, and the increased protein carbonylation could be a result of decreased GSH content.

In the present study, increased level of the activity of enzyme glutathione s-transferase in the liver of mice are under the influence of indoxacarb treatment which might be due GST participates in pollutant detoxification by adding a GSH-group to xenobiotics or their metabolites, so they become more waters soluble and, thus, excreted more easily (Moorhouse
Glutathione-s-transferases are a major family of detoxifying enzymes that catalyze the conjugation of GSH with electrophilic centers of lipophilic substrates, thereby increasing its solubility and aiding their excretion from body depicts the activity of GST in liver of acute and chronic carbofuran-exposed animals (Vontas et al., 2001).

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

The results of the present study revealed that the mice treated with 24, 18 and 12 mg of indoxacarb for 30 days showed a smear of DNA indicates necrosis of the cells in liver of mice. The DNA band was observed in the cells of the liver in the control mice. Similar results were reported that the occurrence of DNA fragmentation is considered to be a hallmark of apoptotic or necrotic cell death caused by hepatotoxins (Ray et al., 1993; Fukuda et al., 1993) and in necrotic lymphoma cell death induced by Ca\textsuperscript{2+} ionophore or hypoxia in mice (Collins et al., 1992; Stachlewitz et al., 1999; Tsutsu et al., 1997). Agarose gel electrophoresis of neuronal cell death showed a smear of DNA due to random cleavage of base pairs during the necrotic process excitotoxic/free radical insults \textit{in vivo} after exposure to peroxynitrite (Emanuela et al., 1995). It has also been suggested that the appearance of DNA fragmentation 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; Ledda-Columbano et al., 1991). 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 liver 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 is aimed to elucidate the indoxacarb induced hepatotoxicity through liver function, histopathology, biochemical contents and Oxidative stress parameters (GSH, TBARS, protein carbonyl and ascorbic acid catalase, SOD, and glutathione S-transferase) activities in mice.

1. Study on the biochemical contents of the liver in female and male mice revealed that, treatment of mice with 18 and 24 mg indoxacarb caused significant decrease in the level of DNA, RNA, protein, glycogen and significant increase in the level of cholesterol. However, mice treated with 6 and 12 mg indoxacarb caused no significant change in the level of biochemical contents, when compared with those of the corresponding parameters of the control mice.

2. Study on the activity of the dehydrogenase, aminotransferase and phosphatase enzymes of the liver in female and male mice revealed that, the treatment with 18 and 24 mg indoxacarb showed significant decrease in the activity of SDH, Na⁺-K⁺ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase and ACP enzymes, whereas the activities of LDH, ASAT, ALAT and AKP enzymes was increased significantly. However, treatment 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.

3. Study on the antioxidants and oxidative stress byproducts of the liver in female and male mice revealed that, treatment of mice with 18 and 24 mg indoxacarb caused significant decrease in the level of GSH and ascorbic acid and significant increase in the level of TBARS and Protein carbonyl. However, mice treated with 6 and 12 mg indoxacarb caused no significant change in the level of biochemical contents, when compared with those of the corresponding parameters of the control mice.

4. Study on the activity of oxidative stress enzymes in female and male mice revealed that, the treatment with 18 and 24 mg indoxacarb the activity of the catalase, SOD, and glutathione s-transferase enzymes was increased significantly. However, treatment with 6
and 12 mg indoxacarb showed no significant change in the activity of enzymes when compared with those of the corresponding parameters of the control mice.

5. The DNA gel electrophoresis analysis in liver revealed that the smear of DNA in the cells of the liver in mice treated with 24, 18 and 12 mg/kg body weight of indoxacarb for 30 days indicates the necrosis of the cells. However, the DNA band was observed normal tissue cells of the liver in the control mice.

Results of the present study clearly indicate that increase in dose indoxacarb treatment caused reduction in protein, glycogen and nucleic acids (DNA and RNA) and increase in cholesterol level in liver and significant increase in ASAT, ALAT, LDH and AKP activities with significant decrease in the activities of SDH, ATPases and ACP enzymes in liver of female and male mice. The alterations in biochemical contents and marker enzymes due to indoxacarb intoxication which is suggestive of an increased tissue synthesis of these enzymes as an adaptive mechanism to chemical stress. In the present study, the enhanced activity of ASAT, ALAT, LDH and AKP a marker enzymes might be due to the cellular damage or increased permeability of plasma membrane. The decrease in DNA, RNA and protein could be attributed to disruption of lysosomal membranes or due to oxidative stress under the effect of indoxacarb and resulted in marked lyses and dissolution of the target materials, DNA, RNA and protein. The elevated lysosomal enzymatic activity accompanied by a decrease in protein and nucleic acid contents in response to indoxacarb with release of enzymes affecting DNA, RNA and protein metabolism. The oxidative stress parameters like protein carbonyl and TBARS were increased due stress and antioxidants like GSH and ascorbic acid were decreased due to oxidative stress and antioxidant enzymes are increased due to stress. The results together with the histopathological observation indicated that indoxacarb caused liver injury.

It is thus concluded from the present work that indoxacarb induced the hepatotoxicity in mice.
### Table 3.1 Effect on liver biochemical contents in female mice after exposure to indoxacarb

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/d)</th>
<th>Biochemical contents (µg/ mg wet weight of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DNA</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>1.79 ± 0.06</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>1.71 ± 0.05</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>1.6 ± 0.04</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>1.55 ± 0.03*</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>1.49 ± 0.04*</td>
</tr>
</tbody>
</table>

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

### Table 3.2 Effect on liver biochemical contents in male mice after exposure to indoxacarb

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/d)</th>
<th>Biochemical contents (µg/ mg wet weight of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DNA</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>1.96 ± 0.05</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>1.79 ± 0.04</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>1.63 ± 0.05</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>1.44 ± 0.04*</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>1.34 ± 0.06*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 10 animals. * Significant P < 0.05 compared to control.
Table 3.3 Effect on liver dehydrogenase, aminotransferase and phosphatase enzymes activity in male mice after exposure to indoxacarb

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/d)</th>
<th>LDH^a</th>
<th>SDH^b</th>
<th>ASAT^a</th>
<th>ALAT^a</th>
<th>Na^+K^+ATPase^c</th>
<th>Mg^++ATPase^c</th>
<th>Ca^++ATPase^c</th>
<th>ACP^d</th>
<th>AKP^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>12.73±0.33</td>
<td>13.10±0.53</td>
<td>15.99±0.45</td>
<td>15.54±0.38</td>
<td>4.08±0.30</td>
<td>6.93±0.40</td>
<td>3.78±0.40</td>
<td>14.40±0.32</td>
<td>15.70±0.46</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>13.01±0.30</td>
<td>12.50±0.45</td>
<td>14.44±0.40</td>
<td>16.03±0.30</td>
<td>5.55±0.24</td>
<td>6.25±0.44</td>
<td>3.28±0.33</td>
<td>14.06±0.35</td>
<td>16.35±0.31</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>17.33±0.25</td>
<td>11.73±0.46</td>
<td>16.99±0.30</td>
<td>16.76±0.35</td>
<td>3.47±0.20</td>
<td>5.61±0.45</td>
<td>2.76±0.20</td>
<td>13.24±0.40</td>
<td>17.20±0.31</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>14.60±0.30</td>
<td>11.03±0.37</td>
<td>17.67±0.35</td>
<td>17.24±0.34</td>
<td>2.99±0.25</td>
<td>4.91±0.40</td>
<td>2.30±0.23</td>
<td>12.25±0.34</td>
<td>18.01±0.36</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>15.73±0.25</td>
<td>10.03±0.45</td>
<td>18.54±0.35</td>
<td>18.03±0.35</td>
<td>2.49±0.22</td>
<td>3.46±0.23</td>
<td>1.80±0.25</td>
<td>11.10±0.33</td>
<td>18.96±0.43</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 5 animals.

* Significant P < 0.05 compared to control.

Table 3.4 Effect on liver dehydrogenase, aminotransferase and phosphatase enzymes activity in male mice after exposure to indoxacarb

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/d)</th>
<th>LDH^a</th>
<th>SDH^b</th>
<th>ASAT^a</th>
<th>ALAT^a</th>
<th>Na^+K^+ATPase^c</th>
<th>Mg^++ATPase^c</th>
<th>Ca^++ATPase^c</th>
<th>ACP^d</th>
<th>AKP^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>13.26±0.35</td>
<td>13.70±0.56</td>
<td>16.18±0.44</td>
<td>14.89±0.40</td>
<td>4.24±0.32</td>
<td>8.01±0.23</td>
<td>3.40±0.35</td>
<td>13.60±0.33</td>
<td>16.53±0.58</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>14.01±0.30</td>
<td>12.96±0.50</td>
<td>16.63±0.42</td>
<td>15.08±0.35</td>
<td>4.10±0.25</td>
<td>7.36±0.25</td>
<td>2.92±0.40</td>
<td>13.20±0.35</td>
<td>17.16±0.48</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>14.70±0.28</td>
<td>12.33±0.45</td>
<td>17.06±0.34</td>
<td>15.99±0.38</td>
<td>3.26±0.30</td>
<td>6.76±0.30</td>
<td>2.38±0.12</td>
<td>12.35±0.44</td>
<td>17.86±0.53</td>
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<tr>
<td>IV</td>
<td>18</td>
<td>15.63±0.25</td>
<td>11.46±0.40</td>
<td>17.99±0.30</td>
<td>16.99±0.31</td>
<td>2.70±0.25</td>
<td>6.30±0.25</td>
<td>1.91±0.24</td>
<td>11.50±0.37</td>
<td>18.93±0.51</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>16.33±0.30</td>
<td>10.50±0.45</td>
<td>18.94±0.42</td>
<td>18.01±0.30</td>
<td>2.3±0.28</td>
<td>5.58±0.21</td>
<td>1.55±0.20</td>
<td>10.85±0.35</td>
<td>19.73±0.56</td>
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</tbody>
</table>

Values are mean ± SEM of 5 animals.

* Significant P ≤ 0.05 compared to control.
Table 3.5 Effect of indoxacarb on liver oxidative stress parameters in female mice

<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 *</td>
<td>TBARS c</td>
<td>Catalase e</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ascorbic acid b</td>
<td>Protein carbonyl d</td>
<td>SOD f</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GST g</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>1.75 ± 0.18</td>
<td>14.00 ± 0.47</td>
<td>0.045 ± 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>430 ± 40.0</td>
<td>1.40 ± 0.10</td>
<td>46.18 ± 0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.83 ± 0.03</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>1.45 ± 0.10</td>
<td>18.3 ± 2.95</td>
<td>0.047 ± 0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400 ± 45.0</td>
<td>1.48 ± 0.15</td>
<td>47.08 ± 0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.88 ± 0.04</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>1.38 ± 0.08</td>
<td>22.20 ± 1.95</td>
<td>0.049 ± 0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>375 ± 30</td>
<td>1.59 ± 0.10</td>
<td>47.90 ± 0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.95 ± 0.02</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>1.30 ± 0.05*</td>
<td>25.6 ± 2.25*</td>
<td>0.055 ± 0.003*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>345 ± 38*</td>
<td>1.65 ± 0.08*</td>
<td>48.55 ± 0.40*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.03 ± 0.02*</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>1.20 ± 0.04*</td>
<td>28.0 ± 2.10*</td>
<td>0.059 ± 0.002*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>320 ± 25*</td>
<td>1.78 ± 0.09*</td>
<td>49.65 ± 0.36*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.08 ± 0.03*</td>
</tr>
</tbody>
</table>

a µmole 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  
e µmole of H₂O₂  
f super oxide dismutase(SOD) unit/mg protein  
g Glutathione-s-transferase(GST)  
mumole/min/mg protein  

Values are mean ± SEM of 10 animals.  

* Significant P ≤ 0.05 compared control.
Table 3.6 Effect of indoxacarb on liver oxidative stress parameters in male mice

<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>1.70 ± 0.20</td>
<td>415 ± 35</td>
<td>16.70 ± 2.15</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>1.59 ± 0.25</td>
<td>400 ± 47</td>
<td>20.90 ± 3.10</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>1.50 ± 0.15</td>
<td>388 ± 50</td>
<td>23.9 ± 1.10</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>1.35 ± 0.10*</td>
<td>350 ± 25*</td>
<td>28.8 ± 2.10*</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>1.23 ± 0.10*</td>
<td>325 ± 30*</td>
<td>32.50 ± 1.50*</td>
</tr>
</tbody>
</table>

- a µmole of glutathione(GSH)/ mg protein
- b ngm of ascorbic acid
- c nmoles thiobarbaturic acid(TBARS)/gm protein
- d nmoles of protein carbonyl/mg protein
- e µmole of H₂O₂
- f super oxide dismutase(SOD) unit/mg protein
- g Glutathione-s-transferase(GST) µmole/min/mg protein

Values are mean ± SEM of 10 animals.

* Significant P ≤ 0.05 compared control.
Table 3.7  Effect on body and organs weight in female mice after exposure to indoxacarb

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/d)</th>
<th>Change in body weight (g)</th>
<th>Relative organs weight / 100 g body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver (g)</td>
<td>Thyroid (mg)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>2.89 ± 0.21</td>
<td>7.56 ± 0.40</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>2.74 ± 0.30</td>
<td>7.12 ± 0.23</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>2.62 ± 0.23</td>
<td>6.54 ± 0.36</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>2.48 ± 0.28*</td>
<td>6.03 ± 0.21*</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>1.97 ± 0.19*</td>
<td>5.33 ± 0.10*</td>
</tr>
</tbody>
</table>

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

Table 3.8  Effect on body and organs weight in male mice after exposure to indoxacarb

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/d)</th>
<th>Change in body weight (g)</th>
<th>Relative organs weight / 100 g body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver (g)</td>
<td>Thyroid (mg)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>2.85 ± 0.28</td>
<td>7.46 ± 0.41</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>2.75 ± 0.34</td>
<td>6.88 ± 0.24</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>2.60 ± 0.23</td>
<td>6.37 ± 0.17*</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>2.40 ± 0.41*</td>
<td>5.69 ± 0.26*</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>1.90 ± 0.32*</td>
<td>5.04 ± 0.36*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 10 animals  * Significant P < 0.05 compared to control.
Graph 3.1. Effect on liver biochemical contents in female mice after exposure to indoxacarb

Graph 3.2. Effect on liver biochemical contents in male mice after exposure to indoxacarb
Graph 3.3. Effect on liver dehydrogenase, aminotransferase and phosphatase enzymes activity in female mice after exposure to indoxacarb.

Graph 3.4. Effect on liver dehydrogenase, aminotransferase and phosphatase enzymes activity in male mice after exposure to indoxacarb.

[Diagrams showing enzyme activity levels and treatments]
Graph 3.6. Oxidative stress parameters of the liver in female mice after exposure to indoxacarb

Graph 3.5. Oxidative stress parameters of the liver in female mice after exposure to indoxacarb

<table>
<thead>
<tr>
<th>Treatment (mg/kg/day)</th>
<th>Control</th>
<th>6</th>
<th>12</th>
<th>18</th>
<th>24</th>
</tr>
</thead>
</table>

* Significant
Graph 3.7. Oxidative stress parameters of the liver in male mice after exposure to indoxacarb

Graph 3.8. Oxidative stress parameters of the liver in male mice after exposure to indoxacarb
Graph 3.9. Effect on body and organs weight in female mice after exposure to indoxacarb

Graph 3.10. Effect on body and organs weight in male mice after exposure to indoxacarb