PUBLICATIONS
Indoxacarb induces testes oxidative stress in Swiss Albino Mice

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Abstract

Indoxacarb: (S)-methyl 7-chloro-2,5-dihydro-2-[(methoxycarbonyl) [4-(3,4-dimethoxyphenyl) amino] carbonyl] indeno(1,2-e) [1,3,4] oxadiazine-4a (3H) carboxylate, is a pyrazoline broad spectrum insecticide. The indoxacarb containing technical formulation was evaluated for its effects on the testes oxidative stress products and enzymes in male albino mice. Normal Swiss albino mice of 90 days old weighing about 25-30g were used in the experiment. The mice were administered with 6, 12, 18, and 24mg/kg body wt indoxacarb for 30 days. The mice administered with distilled water were served as control and mice were sacrificed on day 31st or 24 hours after the terminal exposure. There was an increase in the testis oxidative stress byproducts of Lipid (Malondialdehyde) and protein (Protein carbonyl) contents and decrease in the Glutathione (GSH) and ascorbic acid contents in the mice treated with 18 and 24mg/kg/day indoxacarb. Indoxacarb induced increase in Superoxide dismutase (SOD), Catalase (CAT) and Glutathione-s-transferase (GST) in mice treated with 18 and 24 mg/kg/day. However, there was no change in the oxidative stress byproducts and enzymes in the mice treated with 6 and 12mg/kg/day indoxacarb. The results of the present study suggest that chronic exposure to indoxacarb insecticide has deleterious effect on testes.

Key Words: Testis, Indoxacarb, Antioxidants, Oxidative stress enzymes, Albino mice

Introduction

Oxidative stress is defined as a disruption of the antioxidant balance in favor of the former, leading to potential damage [1]. It is a result of one of three factors: An increase in reactive oxygen species (ROS), impairment of antioxidant defence systems, or in sufficient capacity to repair oxidative damage. Damage induced by ROS included alterations of cellular macromolecular such as membrane lipids, DNA and proteins. The damage may alter all the function through changes in intracellular calcium or intracellular pH and eventually can lead to cell death [2, 3].

It has been reported that 15% of all couples living in the United States have difficulty in conceiving. Male factors are responsible in at least 30% of cases and in another 20% the pathology is found both in men and women. Approximately 6% of men between the age of 15 and 50 suffer from male infertility [4]. A metaanalysis of 61 studies worldwide found a downward trend in sperm count and volume of seminal fluid over the past 50 years [5]. In 1940 the average sperm count was 113 million per ml by 1990 the count had dropped to 66 million per ml [6]. This decreasing trend in sperm count has led to speculation that recent environmental dietary and or life style changes are interfering with a man's ability to produce spermatozoa. It is
believed that these factors exert their detrimental effects through oxidative stress (OS). It has been shown that 1,1,1-trichloro o-2, 2-bis (p-chlorophenyl) ethane (DDT) and related compounds share a mechanism of action similar to pyrethroids. Several studies have demonstrated that DDT and methoxychlor induce oxidative stress and lipid peroxidation [7, 8], adversely affects the male reproductive system, by decreasing the antioxidant enzymes in the epididymal sperm of goats [9], and rats [10, 11, 47]. Many studies have shown the oxidative stress effect of lindane in tests [12].

Indoxacarb is a recently introduced oxadiazine insecticide derived from pyrazoline with activity against a wide range of pests [13]. Indoxacarb is used as a proinsecticide in insects, which requires to be converted by one or more hydrolyses to its N-decarbomethoxylation metabolite (N-decarbomethoxylation JW 062), designated DCJW (Fig. 1) [14]. Several studies have demonstrated that DCJW is effective at blocking sodium channels at this target site [15-18]. However, indoxacarb and DCJW have also been shown to affect mammalian nicotinic acetylcholine receptors [19], and have a weak effect on mammalian (gamma-amino butyric acid) GABA receptors [19]. Therefore, the present investigation was undertaken to study the effect of indoxacarb on antioxidants, oxidative stress byproducts and oxidative stress enzymes in albino mice.

Materials and Methods

Insecticide

The sample of indoxacarb (indoxacarb 14.5%) used in experiments was commercial insecticide supplied by E.I DuPont India Pvt., Ltd., Haryana obtained from the local company’s market containing Indoxacarb (a.i) 14.5 (w/w) in active enantiomer 6% (w/w) amorphous silicon dioxide 7% (w/w) polyethoxylated polyalyl phenol phosphate 6% (w/w) distilled methyl soyate 57.5% (w/w).

![Indoxacarb](image)

**Fig. 1. Structure of indoxacarb and DCJW**

Laboratory bred adult virgin Swiss albino mice were used in the experiments. Mice aged 90 days old weighing between 25-30 g were used. The mice were maintained in the P.G. Department of Studies in Zoology, Karnataka University, Dharwad. Mice breed quite normally, almost throughout the year and permitted through local ethical committee. They were housed in separate polypropylene cages containing sterile paddy husk as bedding material. The mice were provided with standard mice pellet diet “Gold Mohar” (Hindustan Liver Company, Mumbai) and water ad libitum. The mice were maintained under normal day/night schedule (12 L: 12 D) at room temperature 25 ± 2°C.

The doses were given orally in distilled water, below their acute level of intoxication according to their weight. The mice were divided in to 5 groups, 1st group used as control and remaining 4 groups were used for graded dose study. Each group consists of 10
mice. The mice were given 6, 12, 18 and 24 mg/kg body weight indoxacarb for 30 days. Control mice were received distilled water. All mice were autopsied by cervical dislocation on day 31st day or 24 hrs after the terminal exposure.

Oxidative Stress parameters Estimation

Oxidative stress parameters such as estimations of Glutathione (GSH) [20], Ascorbic acid [21], Thiobarbituric acid (TBARS) [22], protein carbonyl [23], Catalase [24], Superoxide dismutase (SOD) [25], and Glutathione-s-transferase (GST) by [26]. Because of its dense negative charge, fluorine has a very strong hydrogen binding capacity and is prone to bind various antioxidants and anti-oxidation enzymes. It has been reported that endosulfan and chlorpyrifos causes the oxidative stress and decrease the amount of GSH in different tissues of rats [37]. In the present study the reason for decreased GSH level in testes, under the influence of indoxacarb treatment in mice may be due to denaturation of indoxacarb or due oxidative stress produced by the compound or may be due to the conjugation of indoxacarb or its metabolites to GSH.

Statistical Analysis

Statistical significance between control and experiment data were subjected to analysis of variance (ANOVA) together with Dunnett test (P<0.05).

Results and Discussion

The present findings revealed that an increase in the dose of indoxacarb showed decrease in the concentration of ascorbic acid. Antioxidant such as ascorbic acid, Vitamin E and GSH protect germ cells against oxidative DNA damage and play an important role in spermatogenesis [38]. Ascorbic acid is a major chain-breaking antioxidant and is present in the extracellular fluid. It neutralizes hydroxyl, superoxide, and hydrogen peroxide radicals and prevents sperm agglutination [39]. In fact, deficiency of ascorbic acid and vitamin E causes the disturbance of spermatogenesis [40, 41]. It has been reported that methyl parathion (MP) is known to induce sperm shape and abnormalities and reduce the sperm count in rodents, possibly mechanism related to reduced ascorbic acid level in the testis [42, 43]. These facts indicate that the defence mechanism against oxidative stress plays critical roles in the maintenance of impaired mitochondrial ROS metabolism. There are findings that consistent with decreased concentration of GSH, and ROS production in the testis of chicks and rodents, exposed to various prooxidant xenobiotics [9, 11, 12, 31-33]. Since indoxacarb is fluorinated compound it has been suggested that oxidative stress is induced in the testes of mice exposed to different doses of fluorine (as NaF), [34-36].

GSH in different tissues of rats [37]. In the present study the reason for decreased GSH level in testes, under the influence of indoxacarb treatment in mice may be due to denaturation of indoxacarb or due oxidative stress produced by the compound or may be due to the conjugation of indoxacarb or its metabolites to GSH.

The present findings revealed that, an increase in the dose of indoxacarb showed decrease in the concentration of ascorbic acid. Antioxidant such as ascorbic acid, Vitamin E and GSH protect germ cells against oxidative DNA damage and play an important role in spermatogenesis [38]. Ascorbic acid is a major chain-breaking antioxidant and is present in the extracellular fluid. It neutralizes hydroxyl, superoxide, and hydrogen peroxide radicals and prevents sperm agglutination [39]. In fact, deficiency of ascorbic acid and vitamin E causes the disturbance of spermatogenesis [40, 41]. It has been reported that methyl parathion (MP) is known to induce sperm shape and abnormalities and reduce the sperm count in rodents, possibly mechanism related to reduced ascorbic acid level in the testis [42, 43]. These facts indicate that the defence mechanism against oxidative stress plays critical roles in the maintenance of impaired mitochondrial ROS metabolism. There are findings that consistent with decreased concentration of GSH, and ROS production in the testis of chicks and rodents, exposed to various prooxidant xenobiotics [9, 11, 12, 31-33]. Since indoxacarb is fluorinated compound it has been suggested that oxidative stress is induced in the testes of mice exposed to different doses of fluorine (as NaF), [34-36]. Because of its dense negative charge, fluorine has a very strong hydrogen binding capacity and is prone to bind various antioxidants and anti-oxidation enzymes. It has been reported that endosulfan and chlorpyrifos causes the oxidative stress and decrease the amount of GSH in different tissues of rats [37]. In the present study the reason for decreased GSH level in testes, under the influence of indoxacarb treatment in mice may be due to denaturation of indoxacarb or due oxidative stress produced by the compound or may be due to the conjugation of indoxacarb or its metabolites to GSH.
spermatogenesis and prevention of testicular atrophy. It has been reported that depletion of GSH in lung and kidney may in part contribute to the decrease in ascorbic acid observed following 0.15 ng 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) /kg /day exposure [44]. It has been also shown that reduced ascorbic acid level may be due to intoxication of di (2-ethylhexyl) phthalate (DEHP) in germ cells [45]. In the present study the reason for decreased concentration of ascorbic acid may be due to production of reactive oxygen species or due to its antioxidant property of ascorbic acid.

The present findings revealed that an increase in the dose of indoxacarb showed increase in the concentration of lipid peroxidation. Lipid peroxidation (LPO) is a chain reaction between polyunsaturated fatty acids and ROS, and it produces lipid peroxides and hydrocarbon polymers that are both highly toxic to the cell. Malondialdehyde is an end product of peroxidation of polyunsaturated fatty acids and related esters, and is, therefore, used as a marker of lipid peroxidation. It has been reported that hydrogen peroxide treatment induced a significant increase in the lipid peroxidation and enhanced ROS generation in testes after 1- and 2-week exposure, clearly suggesting their potential to induce significant oxidative stress in the reproductive system of rats results are consistent with the earlier data on hydrogen peroxide (HP) induced oxidative damage in rat [47, 48], and rats [49]. It has been also shown that increase in LPO was evident after 1-week exposure [54]. These findings are consistent with elevated lipid peroxides, malondialdehyde, and ROS production in the testis mitochondria of adult rodents exposed to various prooxidant xenobiotics [7, 8]. It is reported that F in the testis of mice causes an increase in MDA. It is reported that OP pesticides induce oxidative stress. It has been observed that testicular damage caused by quinalphos was due to free radical-mediated by increased LPO [50]. It is reported that lindane causes oxidative stress in the testes and increases lipid peroxidation [51]. It has been reported that chlorpyrifos, paraxquat and diquat causes the oxidative stress the increase in MDA in different tissues of rats [52, 53]. In the present study the reason for increased MDA level in testes under the influence of indoxacarb treatment in mice might be caused due to the conjugation of indoxacarb or its metabolites to the polyunsaturated fatty acids or by production of ROS reacts with polyunsaturated fatty acids or accumulation of lipophilic components of pesticides conjugated with the fatty acids.

The present findings revealed that an increase in the concentration of protein carbonyl. Among the various oxidative modifications of amino acids in proteins, protein carbonyl formation may be an early biomarker of ROS-mediated protein oxidation [54]. It has been reported that protein carbonyls in testis were markedly increased in rats treated with higher doses of HP at week 2. While tertiarybutyl hydrogen peroxide (tBHP) treatment enhanced carbonyl content by 40% and 78% over the control levels, cumene hydrogen peroxide (cHP) treatment increased the carbonyl content by 35% and 50% at the higher doses [46]. Evidence of HP-induced oxidative damage in testis was evident from the elevated levels of protein carbonyls at higher doses. Accumulation of high amounts of carbonyls in testis after 2-week HP exposure reflects a high rate of protein oxidation, [46]. In the present study the reason for increased protein carbonyl level in testis under the influence of indoxacarb treatment in mice might be due to production of ROS or oxidative stress.

The present findings revealed that an increase dose of indoxacarb showed increase
in the activity of catalase and SOD. In the present model, HP treatment significantly enhanced the activities of various antioxidant enzymes in testis. The concomitant increase in the activities of catalase (CAT) and Glutathione peroxidase (GPX) suggests that HP may increase the level of hydrogen peroxide, the substrates for these enzymes. It has been reported that DEHP enhanced the generation of ROS in testicular cells Cu/Zn-SOD and catalase were increased [45]. It is reported that lindane causes oxidative stress in the testes and increase in catalase and SOD activity might be due to higher levels of SOD and catalase following adaptation might have protected the testes from more severe injury due to oxidative stress. Recently it has been reported that due to intoxication of diazinon an increase in testicular superoxide dismutase (SOD) activity was detected on 32 day [55]. It has been reported that administration of methoxychlor decreased the activities of catalase, superoxide dismutase[11]. Similar changes have also been reported in the epididymis of rats treated with methoxychlor. In the present study the reason for increased activity of catalase and SOD under the influence of indoxacarb treatment in mice may be due to the production of reactive oxygen species or may be due to production of H2O2 in order to be eliminated from the tissues or H2O2 byproduct is further converted to non toxic product in the tissues.

The present findings revealed that, an increase in dose of indoxacarb showed increase in the activity of GST. GSTs are a group of primary phase II detoxification enzymes that provide protection against products of oxidative stress whose abundance and protective role in germ cells has been adequately demonstrated [56-58]. It has been suggested that the marked increase in the activity of GST observed in the current study is consistent with the earlier findings in vitro suggesting its vital role under HP intoxication [30, 59]. It has been shown that increase in the activity of GST due to organochlorine contaminants indeed, GST participates in pollutant detoxification by adding a GSH-group to xenobiotics or their metabolite, so they become more water-soluble and, they are excreted more easily [60]. In the present study the reason for increased activity of Glutathione S-transferase under the influence of indoxacarb treatment in mice testis may be due to ROS produced by the indoxacarb or to defexify the pesticide or in order to eliminate the pesticide from the body by conjugation with the GSH to become more water-soluble.

Acknowledgements

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REFERENCES

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Table 1. Effect of indoxacarb on testes oxidative stress parameters in albino 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>1.75 ± 0.20</td>
<td>300 ± 25</td>
<td>0.65 ± 0.25</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>1.70 ± 0.10</td>
<td>290 ± 10</td>
<td>0.75 ± 0.10</td>
</tr>
<tr>
<td>III</td>
<td>12</td>
<td>1.55 ± 0.05</td>
<td>250 ± 15</td>
<td>0.85 ± 0.05</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>1.43 ± 0.08*</td>
<td>200 ± 30*</td>
<td>1.29 ± 0.13</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>1.30 ± 0.10*</td>
<td>172 ± 14*</td>
<td>1.75 ± 0.18</td>
</tr>
</tbody>
</table>

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

Indoxacarb induces testes oxidative stress in Swiss Albino Mice
Indoxacarb induces testes oxidative stress in Swiss Albino Mice
Dear Dr. B. B. Kaliwal,

You are most respectfully invited to participate in International Congress of Chemistry and Environment ICCE-2009 Conference at Thailand from 21st Jan. 2010 to 23rd Jan. 2010. Conference will be held in association with Ubonratchathani University and PACCON (The Pure and Applied Chemistry International conference-organized by Chemical Society of Thailand). The conference venue is at Sunee Grand Hotel and Convention Center, Ubonratchathani, Thailand.

We are pleased to inform you that your abstract/paper entitled “Indoxacarb induces hepatic toxicity in Swiss albino mice” has been accepted by scientific committee for oral presentation in the conference. Your abstract/paper will be published in book of proceedings which will be a special issue of our peer reviewed international quarterly journal “Research Journal of Chemistry and Environment” (ISSN 0972-0626). Our journal is indexed and abstracted in Chemical Abstracts, Science Citation Index Expanded (SciSearch®), SCOPUS and Journal Citation Reports/Science Edition. We are publishing the journal regularly since last 13 years.


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With kind regards and best wishes,

Yours sincerely,

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Indoxacarb induces hepatic toxicity in Swiss albino mice

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Abstract

Indoxacarb : (S)-methyl 7-chloro-2,5-dihydro-2-[[methoxycarbonyl] [4-(trifluoromethoxy)phenyl]amino]carbonyl]inden[1,2-e][1,3,4]oxadiazine-4a(3H) carboxylate, is a pyrazoline broad spectrum insecticide. The indoxacarb containing technical formulation was evaluated for its effect on the liver histopathology, biochemical contents and enzymes activity in albino mice. Normal 90 days old Swiss albino mice, weighing about 25-30 g were used in the experiment. The mice were administered orally with 6, 12, 18, and 24 mg/kg body wt indoxacarb for 30 days. The mice administered with distilled water served as control and mice were sacrificed on day 31st or 24 hours after the terminal exposure. Liver dissected out were freed from adherent tissue and weighed to the nearest milligram. Mice treated with 6 and 12 mg/kg/day indoxacarb for 30 days showed normal structure of the liver. Mice treated with 18 and 24 mg/kg/day indoxacarb revealed that hepatocytes adjacent to the central vein were spared dilation of central vein and sinusoids between hypertrophied hepatocytes. Liver biochemical contents showed that the levels of DNA, RNA, protein and glycogen were decreased but there was an increase in the level of cholesterol in the mice treated with 18 and 24 mg/kg/day indoxacarb. The mice treated with 18 and 24 mg/kg/day of indoxacarb caused a decrease in the enzymes activity of SDH (Succinate dehydrogenase), Na⁺-K⁺ ATPase, Mg²⁺ATPase, Ca²⁺ ATPase, ACP (Acid phosphatase) and increase in LDH (Lactate dehydrogenase), ASAT (Aspartate aminotransferase), ALAT (Alanine aminotransferase) and AKP (Alkaline phosphatase). The results of the present study suggest that chronic exposure to indoxacarb insecticide has deleterious effect on liver. Further the study also revealed that the indoxacarb might have affected the cell metabolism.

Key words: Indoxacarb, liver, histology, biochemical contents, toxicity, mice.
Introduction

Pesticides are used for the welfare of human beings but with time, they will challenge us by showing their toxicity. They can be directly exposed to us or indirectly through food chain. Indiscriminate use of pesticides is on increase. Indoxacarb is a recently introduced oxadiazine insecticide derived from pyrazoline with activity against a wide range of pests. In insects, indoxacarb appears to be decarbomethoxylated to DCJW by an esterase/amidase. Several studies have demonstrated that DCJW is effective at blocking sodium channels at this target site. However, indoxacarb and DCJW have also been shown to affect mammalian nicotinic acetylcholine receptors and have a weak effect on mammalian GABA receptors.

The 8-week rat liver assay uses as endpoint the development of altered foci of hepatocytes (AFH), which express the placental form of the enzyme glutathione s-transferase (GST-P+foi) and have been considered as early indicators of the rat liver carcinogenic process due to toxicity induced by the diuron. Nuvacron into mice gave high incidence of structural and numerical chromosomal aberrations in both somatic and germ cells of males liver and embryos of pregnant females. Studied on nucleic acid and protein profile in normal and malnourished rat liver on exposure to organophosphorous group of pesticides that were affected in both. Mancozeb and in other study carbosulfan treatments have altered the levels of protein, glycogen and total lipids in liver, uterus and ovary in intact and hemicastrated rats and mice. Transaminases, ACP and AKP activities were increased in plasma, liver, kidney, lung, brain, heart, intestine and muscle of rat treated with dichlorvos. Similar reports were observed due to intoxication of endosulfan indicated hepato-nephrotoxicity. Dimethoate an organophosphorous is found to have affected the protein and carbohydrate metabolism as well as transaminases in the liver tissue of the fish *Clarias batrachus* (Linn) and made alteration in protein metabolism of the muscle tissue in the same fish. Therefore, the present investigation was carried out to evaluate the effect of indoxacarb on histologic, biochemical contents and enzymes activity of the liver in albino mice.
Materials and methods

Insecticide

The sample of indoxacarb (indoxacarb 14.5%) (S)-methyl7-chloro-2,5-dihydro-2-(methoxycarbonyl)[4-(trifluoromethoxy)phenyl]amino]carbonyl]indeno[1,2-e][1,3,4]oxadiazine-4a(3H) carboxylate) used in experiments was commercial insecticide supplied by E.I DuPont India Pvt., Ltd., Haryana obtained from the local company's market containing indoxacarb (a.i) 14.5 (w/w) in active enantiomer 6% (w/w) amorphous silicon dioxide 7% (w/w) polyethoxylated polyalyl phenol 9%(w/w) polyethoxylated polyalyl phenol phosphate 6%(w/w) distilled methyl soyate 57.5%(w/w).

![Structural Formula of indoxacarb (C_{22}H_{17}ClF_3N_3O_7)](image)

Animals

Laboratory bred adult virgin female Swiss albino mice were used in the experiment. Mice aged 90 days old weighing between 25-30 g were used. The mice were maintained in the P.G. Department of Studies in Zoology, Karnataka University, Dharwad. Mice breed quite normally, almost throughout the year and permitted through local ethical committee. They were housed in separate polypropylene cages containing sterile paddy husk as bedding material. The mice were provided with standard mice pellet diet “Gold Mohar” (Hindustan Liver Company, Mumbai) and water ad libitum. The mice were maintained under normal day/night schedule (12 L: 12 D) at room temperature 25 ± 2°C.
Treatments

The doses were given orally in distilled water, below their acute level of intoxication according to their body weight. The mice were divided into 5 groups, 1st group was used as control and remaining 4 groups were used for graded dose study. Each group consists of 10 mice. The mice were given 6, 12, 18 and 24 mg/kg body weight indoxacarb for 30 days. Control mice were received distilled water. All mice were autopsied by cervical dislocation on 31st day or 24 hrs after the terminal exposure. The liver of all mice was dissected out and in each group mice were processed for histopathological, biochemical contents and enzymes activity study.

Histologic studies

For Histologic study, freshly removed liver was fixed in Bouin's fluid, dehydrated in ethanol, embedded in paraffin and serial sections at 5 μm were prepared and stained with haematoxylin and eosin.

Biochemical estimations

The biochemical study such as estimation of DNA and RNA carried out as per the method described by Schneider15., Protein by Lowry et al16., Glycogen by Carrol et al17., Cholesterol by Abell et al18., activities of enzymes such as lactate dehydrogenase (LDH) by King19., succinate dehydrogenase (SDH) by Nachlas et al20., aspartate amino transferase (ASAT) and alanine amino transferase (ALAT) by Yatzidis21, Na⁺-K⁺ ATPase, Ca⁺⁺ ATPase, Mg⁺⁺ ATPase were assayed according to the method described by Jinna et al22., acid phosphatase (ACP) and alkaline phosphatase (AKP) by method of Bergmeyer and Bernt23.

Statistical analysis

Statistical significance between the control and experimental data were subjected to analysis of variance (ANOVA) together with Dunnett's test (P<0.05).
Results and discussion

Histologic studies

In the present study, liver histologic observations of the control mouse showed radially arranged hepatic cords around the central vein and the hepatocytes with centrally located nuclei (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 (Figs. 2, 3). 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 (Figs. 4, 5).

Similar results have been observed that, treatment with endosulfan (10 mg/kg/ day) in rats caused liver damage which includes dilation of sinusoidal spaces with irregular nuclear shape, degenerative changes with binucleated cells, hypertrophy of hepatocytes and lymphocytic infiltration in central vein. 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. Shivanandappa and Krishnakumari 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. Methyl demeton, an organophosphate insecticide is known to cause degenerative changes in hepatocytes causing necrosis and cytoplasmolysis in rats. Further the dilation and congestion of sinusoids, ballooning of hepatocytes with pyknotic nuclei and focal necrosis was found in rats.

Biochemical contents

In the present graded dose study, mice treated with 18 and 24 mg/kg/day indoxacarb for 30 days showed decrease in DNA, RNA, protein, glycogen and increase in the level of cholesterol. There was no change in biochemical contents of the group treated with 6 and 12 mg/kg/day indoxacarb for 30 days in mice. Hence, in the present investigation, a significant
decrease in the levels of DNA and RNA contents in the liver of mice were found with higher dosage. Similar results have been reported in carbosulfan, methomyl and phosphomidon intoxication with decrease in nucleic acid contents in kidney and liver\textsuperscript{28-30}. It has been reported that with nuvaron 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\textsuperscript{8}. The same reduction was obtained for liver-RNA content in both male and pregnant female mice by nuvaron treatment\textsuperscript{8}. The reduction in DNA was also observed by several authors who attributed this effect to the inhibition of DNA synthesis or DNA damage by carbaryl\textsuperscript{31}. The malathion was also found to be genotoxic and inducing severe DNA lesions\textsuperscript{32}. 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\textsuperscript{33}, inhibition of its synthesis\textsuperscript{34} or to the general inhibition of DNA dependent RNA polymerase\textsuperscript{35} this may be the reason for reduction in DNA and RNA contents. It has been indicated that 2,4-D-CoA may contribute to 2,4-D-protein adduct formation \textit{in vivo} and therefore, associates in hepatotoxicity\textsuperscript{36}. In mammalian cells \textit{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\textsuperscript{37}. Abdel-Basset and Zaki\textsuperscript{38} have 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\textsuperscript{39} to induce RNA reduction in liver cells of guinea pig. In the present study, the decreased levels of nucleic acids of the liver of mice were under the influence of indoxacarb treatment may be due to genotoxic effect, inducing severe DNA lesions\textsuperscript{32} or formation of chromosomal breaks by breaking the phosphodiester backbone of DNA molecule\textsuperscript{40}.

In the present investigation the decreased levels of protein and glycogen contents in the liver of mice were found with higher dosage. It has been reported that protein 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)\textsuperscript{41} this may be one of the reason for decrease in protein content. It has been suggested that the protein damage may be the result of direct impact of 2,4-D or its indirect effect, due to generation of free radicals (protein peroxidation)\textsuperscript{41}. Sancho \textit{et al.},\textsuperscript{42} 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. 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 which recorded a decrease in liver glycogen level after administration of carbamate 43,44. The same data were observed by Dekundy et al. 45 which indicated an enhanced rate of glycolysis due to carbamate stress, this may be the one of the reason for decrease in glycogen content.

In the present investigation the increased level of cholesterol contents in liver of mice are found with higher dosage. 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 46 this may be one of the reason for the increase in the level of cholesterol. Recently it has been reported by Devendra et al. 47 due to intoxication of carbofuran and cartap the elevation in total serum cholesterol level observed could be due to blockage of liver bile ducts causing reduction or cessation of its secretion to the duodenum subsequently causing cholestasis. The disruptions of formation of lipoprotein have been reported by Hassan et al. 48 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 to be the precursor of cholesterol biosynthesis this may be one of the reason for the increased level of cholesterol 47. It has been also reported that intoxication of carbofuran resulted in the elevation of serum total cholesterol level Gupta et al. 49. It has been reported that mancozeb and carbofuran treatments have altered levels of protein, glycogen and total lipids in liver, uterus and ovary in intact and hemicastrated rats and mice 50,51. Diethyl dithiocarbamate inhibits hepatic cyt-p450 dependent activity in rats 52. In the present study the increase in the cholesterol level of the liver in mice may be due to inhibitory action of pesticide on Cyt-p450 enzyme or high affinity binding, thereby affecting the enzymes which are essential for cholesterol break up causing deposition of cholesterol in the tissue 53.

Enzymes activity

In the present graded dose study, mice treated with 18 and 24 mg/kg/day indoxacarb for 30 days showed decrease in the SDH, Na+ - k+ ATPase, Mg++ ATPase, Ca++ ATPase, ACP and increase in the activity of LDH, ASAT, ALAT, and AKP. There was no change in the enzymes activity in the mice treated with 6 or 12 mg/kg/day indoxacarb for 30 days in mice. These findings revealed that, increase in dose exposure of indoxacarb showed increase in LDH, ASAT
and ALAT and decrease in the activity of SDH in the liver of albino mice. It has been observed that 7, 12-dimethylbenz (a) anthracene (DMBA) induced increase in the activity of hepatic and renal LDH may be attributed to the enhanced enzyme synthesis. The increase in the LDH level indicate that the energy demands are met by anaerobic respiration through increase in LDH activity, may be one of the reason for increased level of LDH. It has been suggested that the stressed animals are meeting its energy requirements through anaerobic oxidation. Rady et al., 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 has reported that significant decrease in the activity of liver SDH suggests that anaerobic metabolism was favored over aerobic oxidation of glucose through Krebs cycle in order to mitigate the energy crisis for survival.

The elevation in transaminases activity that was noticed in carbamate treated suggests the existence of heavy drain during carbamate stress, which is known to induce elevation of serum transaminases. From another point of view, elevations of transaminases activity in blood have been considered as indicator of tissue damage, without any specific damage any 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. Srivastava et al., 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. Similarly, increased levels of plasma ASAT and ALAT has been reported on treatment with quinolphos in Buffalo calves. The increase in the activity of enzymes may show that the protein was taken as an alternative source of energy, due to high energy demand that is induced by pesticide intoxication. This result is confirmed by the marked rise of ASAT and ALAT activities reported in the present study. The enhanced activities of transaminases induced tissue proteolysis. This phenomenon is previously recorded for different fish species subjected to pesticides.

The present findings revealed that, increase in dose of exposure to indoxacarb caused decrease in the activity of Na⁺-K⁺ATPase, Mg⁺⁺ATPase and Ca⁺⁺ATPase in liver of mice.
Ksheerasagar and Kaliwal\textsuperscript{28} have reported that carbosulfan treated albino mice showed significantly decreased activity of ATPases and ACP in mouse liver and kidney. Similar results were obtained due to intoxication of methomyl and phosphomidon\textsuperscript{29,30}. It has been reported that N--methyl carbamate inhibits ATPase system and alters calcium, magnesium homeostasis or energy related metabolic alterations\textsuperscript{64}. Thus, the results obtained from the present observation indicate that, the indoxacarb caused inhibition of cell membrane Na\textsuperscript{+}-K\textsuperscript{+}ATPase, an important enzyme utilizing the energy from ATP hydrolysis for transport of several actions.

The present findings revealed that, increase in dose of exposure of indoxacarb caused decrease in ACP and increase in AKP activity in liver of mice. Similar results have been reported with different pesticides. 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\textsuperscript{12}. Negoha et al.,\textsuperscript{65} have reported that the serum ACP and AKP were elevated whereas kidney and liver ACP and AKP were reduced in chloroquine treated rats. The decrease in ACP activity by pesticides as in the present study probably indicates an altered transport of phosphate\textsuperscript{66} and inhibitory effect on the cell growth and proliferation\textsuperscript{67}. The elevated AKP activity in liver which is suggestive of an increase in tissue synthesis of these enzymes acts as an adaptive mechanism to chemical stress\textsuperscript{68}.

Acknowledgements

The authors are grateful to the Post-Graduate Department of Studies in Microbiology and Biotechnology, Karnatak University, Dharwad for providing the necessary facilities.

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EXPLANATION TO PHOTOMICROGRAPHS

Fig 1. T. S. of the liver of the control mouse showing radially arranged hepatic cords around the central vein. Normal hepatocytes with centrally located nuclei.

Fig 2 & 3. T. S. of the liver 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).

Fig 4. T. S. of the liver 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 5. T. S. of the liver 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 lost.

Photographs original exposure at × 100

Abbreviations: V - Vacuoles, CV - Central vein, H - Hepatocytes, HH - Hypertrophied hepatocytes, PN - Pyknotic nuclei.
Effect on liver biochemical contents in female albino mice after exposure to indoxacarb

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
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<th>3</th>
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<th>6</th>
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<tr>
<td>Cholesterol (mg/dl)</td>
<td>10.28 ± 0.16</td>
<td>10.01 ± 0.19</td>
<td>9.85 ± 0.18</td>
<td>9.79 ± 0.16</td>
<td>9.74 ± 0.15</td>
<td>9.69 ± 0.14</td>
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<td>Protein (mg/dl)</td>
<td>2.45 ± 0.40</td>
<td>3.85 ± 0.32</td>
<td>5.07 ± 0.39</td>
<td>5.86 ± 0.45</td>
<td>6.30 ± 0.43</td>
<td>6.90 ± 0.46</td>
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<td>DNA (pg/mg)</td>
<td>140.40 ± 6.40</td>
<td>156.00 ± 5.70</td>
<td>165.6 ± 7.09</td>
<td>180.00 ± 6.39</td>
<td>188.8 ± 5.09</td>
<td>197.4 ± 6.89</td>
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<tr>
<td>RNA (pg/mg)</td>
<td>3.19 ± 0.09</td>
<td>3.42 ± 0.07</td>
<td>3.60 ± 0.08</td>
<td>3.80 ± 0.09</td>
<td>4.00 ± 0.09</td>
<td>4.20 ± 0.10</td>
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<tr>
<td>Glycogen (mg/g)</td>
<td>13.85 ± 0.20</td>
<td>12.46 ± 0.18</td>
<td>11.97 ± 0.19</td>
<td>11.58 ± 0.20</td>
<td>11.20 ± 0.21</td>
<td>10.81 ± 0.22</td>
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<tr>
<td>DNA (pg/mg)</td>
<td>140.40 ± 6.40</td>
<td>156.00 ± 5.70</td>
<td>165.6 ± 7.09</td>
<td>180.00 ± 6.39</td>
<td>188.8 ± 5.09</td>
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<td>RNA (pg/mg)</td>
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<td>11.20 ± 0.21</td>
<td>10.81 ± 0.22</td>
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Values are mean ± SEM of 10 animals * Significant P 0.05 compared to control.

Table 1

Biochemical contents (mg/dl and g/mg wet weight of tissue)

Effect on liver biochemical contents in female albino mice after exposure to indoxacarb.
Table 2: Effect on liver dehydrogenase, aminotransferase and phosphatase enzymes activity in female albino mice after exposure

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg/d)</th>
<th>Enzyme activity (pmoles / min/ g tissue weight)</th>
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<tr>
<td></td>
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<td>Liver dehydrogenase</td>
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<td></td>
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<td>(a) Number of p-nitrophenol formed</td>
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<td>(min/ g tissue)</td>
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</table>

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

Enzyme activity (a) formed / min / tissue weight.
Dr. Gangadhar  M.Sc., Ph.D., FZSI, FSESc.
Professor and Head Dept. of Bio-technology &
Executive Committee Member
Indian Science Congress Association

Ref. Date: 9-5-2009

National Symposium on
Role of Life Sciences in Climate Change & Global Warming.
INDOXACARB INDUCES RENAL TOXICITY IN SWISS ALBINO MICE

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Abstract

Indoxacarb : (S)-methyl 7-chloro-2,5-dihydro-2-[[[(methoxycarbonyl) [4-(trifluoromethoxy)phenyl]amino]carbonyl]inden[1,2-e][1,3,4]oxadiazine-4a(3H) carboxylate, is a pyrazoline broad spectrum insecticide. The indoxacarb containing technical formulation was evaluated for its effects on the kidney biochemical contents and histopathology in male albino mice. Normal 90 days old Swiss albino mice, of weighing about 25-30g were used in the experiment. The mice were administered 6, 12, 18, and 24mg/kg body wt indoxacarb for 30 days. The mice administered with distilled water served as control and mice were sacrificed on day 31st or 24 hours after the terminal exposure. Kidney dissected out freed from adherent tissue and weighed to nearest milligram and the Kidney histology, estimations of biochemical contents and enzyme activities were carried out. Kidney biochemical contents showed that levels of DNA, RNA, protein and glycogen were decreased but there was an increase in the level of cholesterol in the mice treated with 18 and 24 mg/kg/day indoxacarb. The mice treated with 18 and 24 mg/kg/day of indoxacarb caused a decrease in the enzyme activities of SDH (Succinate dehydrogenase), Na⁺-K⁺ ATPase, Mg⁺⁺ATPase, Ca⁺⁺ATPase, ACP (Acid phosphatase) and increase in LDH (Lactate dehydrogenase), ASAT (Aspartate aminotransferase), ALAT (Alanine Aminotransferase) and AKP (Alkaline phosphatase). The histological study of kidney of mice treated with 18 and 24 mg/ kg/d indoxacarb showed flattened tubular cells and formation of vacuoles and the glomerulus were small, atrophied and loosely attached to Bowman’s capsule. Vacuoles are found prominently due to loss of glomerulus. However, there no significant change in biochemical contents and histologic changes in the kidney of mice treated with 6 and 12 mg/kg/d indoxacarb. The results of the present study suggest that chronic exposure to indoxacarb insecticide
has deleterious effect on kidney. The study also revealed that the indoxacarb might have affected the cell metabolism.

Key words: Indoxacarb, Kidney, Histology, Biochemical contents, Toxicity, Mice.

Introduction

Indoxacarb is a recently introduced oxadiazine insecticide derived from pyrazoline with activity against a wide range of pests. In insects, Indoxacarb appears to be decarbomethoxylated to DCJW by an esterase/amidase. Several studies have demonstrated that DCJW is effective at blocking sodium channels at this target site. However, indoxacarb and DCJW have also been shown to affect mammalian nicotinic acetylcholine receptors and have a weak effect on mammalian GABA receptors.

An organophosphate insecticide malathion in human lymphocytes, and carbamate insecticide carbosulfan affects DNA and RNA metabolism in bone marrow cells and in liver. Dimethoate an organophosphorous is found to have affect on protein and on the carbohydrate metabolism as well as transaminases in the liver tissue of the fish claries batrachus (Linn) and to make alteration in protein metabolism of the muscle tissue in the same fish. Studied nucleic acid and protein propel in normal and malnourished rat liver on exposure to organophosphorous group of pesticides that were affected in both. Mancozeb and in other study carbosulfan treatments have altered the levels of protein, glycogen and total lipids in liver, uterus and ovary in intact and hemicastrated rats and mice. Transaminases, ACP and AKP were increased in plasma, liver, kidney, lung, brain, heart, intestine and muscle of rat treated with dichlorvos, similar reports were observed due to intoxication of endosulfan indicates hepato-nephrotoxicity of endosulfan. Methomyl on hydrolysis gives S-methyl-N-hydroxy thioacetamide, which is rapidly broken down to CO₂ and acetonitrile in rat tissues. Methomyl is potent genotoxic and is capable of inducing structural and numerical chromosomal aberration in mammalian cells. Even methomyl formulation Lannate- 25 induces DNA damage in liver and kidney due to formation of reactive oxygen species in mammals. There are no reports on the effect of indoxacarb on
biochemical contents in albino mice, therefore the present investigation was carried out to evaluate its effects on histologic and biochemical contents of the kidney in albino mice.

**Materials and Methods**

**Insecticide:** The sample of indoxacarb (indoxacarb 14.5%) used in experiments was commercial insecticide supplied by E.I DuPont India Pvt., Ltd., Haryana obtained from the local company's market containing Indoxacarb (a.i) 14.5 (w/w) in active enantiomer 6% (w/w) amorphous silicon dioxide 7% (w/w) polyethoxylated polyalyl phenol 9%(w/w) polyethoxylated polyalyl phenol phosphate 6%(w/w) distilled methyl soylate 57.5%(w/w).

![Structural Formula of Indoxacarb](image)

**Animals and Treatments:** Laboratory bred adult virgin female Swiss albino mice were used in the experiments. Mice aged 90 days old weighing between 25-30g were used. The mice were maintained in the P.G. Department of Studies in Zoology, Karnataka University, Dharwad. Mice breed quite normally, almost throughout the year and permitted through local ethical committee. They were housed in separate polypropylene cages containing sterile paddy husk as bedding material. The mice were provided with standard mice pellet diet “Gold Mohar” (Hindustan Liver Company, Mumbai) and water.
The mice were maintained under normal day/night schedule (12 L: 12 D) at room temperature 25 ± 2°C.

The doses were given orally in distilled water, below their acute level of intoxication according to their weight. The mice were divided into 5 groups, 1st group was used as control and remaining 4 groups were used for graded dose study. Each group consists of 10 mice. The mice were given 6, 12, 18 and 24 mg/kg body weight indoxacarb for 30 days. Control mice were received distilled water. All mice were autopsied by cervical dislocation on 31st day or 24 hrs after the terminal exposure. The kidney of all mice was dissected out and in each group mice were processed for biochemical and for histopathological study.

**Histological studies:** For Histological study, freshly removed kidney was fixed in Bouin’s fluid, dehydrated in ethanol and embedded in paraffin, and serial sections at 5 μm were prepared and stained with haematoxylin and eosin.

**Biochemical Studies:** The biochemical study such as estimation of DNA and RNA carried out as per the method described by Schneider et al., Protein by Lowry et al., Glycogen by Carrol et al., Cholesterol by Abell et al., activities of enzymes such as SDH by Nachlas et al., LDH by King, ASAT and ALAT by Yatzidis, Na⁺-K⁺ ATPase, Ca⁺⁺ ATPase, Mg⁺⁺ ATPase were assayed according to the method described by Jinna et al., ACP and AKP by method of Bergmeyer and Bernt.

**Statistical analysis:** Statistical significance between the control and experimental data were subjected to analysis of variance (ANOVA) together with Dunnett’s test (P<0.05).

**Results and Discussion**

**Histological studies:** Histological studies of kidney control mice showed that normal arrangement of cortical tubules and thick epithelial cells with prominent glomerulus in Bowman’s capsule. The histology of kidney of mice treated with 6 and 12 mg/kg/day...
for 30 days showed loss of normal arrangement of cortical tubules, hypertrophied tubular
with vacuole formation. In mice treated with 18 and 24 mg/kg/day for 30 days showed
enlarged lumen, flattened tubular cells, formation of more vacuoles with loss of
glomerulus and some small atrophied glomerulus loosely attached within Bowman's
capsule.

Biochemical Studies: In graded dose study the mice treated with 18 and 24 mg/ kg/day
indoxacarb for 30 days showed decrease in DNA, RNA, Protein, Glycogen and increase
in the level of cholesterol, There was no change of biochemical contents in the mice
group treated with 6 mg and 12 mg/kg/day indoxacarb for 30 days.

Effect on enzyme activities: In graded dose study the mice dosed with 18 and 24
mg/kg/day for 30 days indoxacarb showed decrease in the SDH, Na\(^+\) - k\(^+\) ATPase, Mg\(^++\)
ATPase, Ca\(^++\) ATPase, ACP and increase in the activity of LDH, ASAT, ALAT, and
AKP. There was no change in the levels of enzyme activities in the mice group treated
with 6 mg and 12 mg/kg/day indoxacarb for 30 days.

In the present investigation the decreased levels of biochemical contents in kidney
of mice are found with higher dosage. Similar reports have been reported that carbamates
insecticide carboxulfan 48 mg/kg/day for 20 and 30 days inhibited synthesis of DNA,
RNA, Protein and glycogen in liver\(^{25}\) and Kidney\(^{26}\). Similarly methomyl intoxication has
reported the decrease in nucleic acid contents in kidney and liver\(^{27}\), organophosphate
pesticide phosphomidon in kidney and liver\(^{28}\). The indoxacarb may induce DNA damage
in liver and kidney with formation of reactive oxygen species. In the present study the
reason for decreased nucleic acids level in kidney under the influence of indoxacarb
treatment in mice might caused genotoxic action by decreased mitotic index and might
disturbed cell division\(^{15,29}\) or might due to cell damage by increased production of ROS
(Reactive Oxygen Species) in both intra and extra cellular spaces, resulting in increased
oxidative stress\(^{30,31,33}\).

In the present study it was found that indoxacarb caused decrease in the level of
total protein and glycogen in the kidney of mice during dose treatment. It has been also
reported that the decrease in total proteins and soluble proteins indicate their hyper
metabolic utilization\(^{34}\). Similar results have been reported that the protein and glycogen
level in testis, liver and kidney was significantly decreased in mice treated with carbosulfan and mancozeb\textsuperscript{25,35,36}, and also hemi castrated rats treated with mancozeb reported significantly decreased level of glycogen and protein in ovary, uterus, and liver\textsuperscript{37,38,39}. Decrease in total protein level might be due to catabolism of protein\textsuperscript{40} or due to increased ROS, which cause damage to protein and glycogen synthesis\textsuperscript{41,42}.

In the present study with higher dose of indoxacarb caused increase in the level of cholesterol in the kidney of mice. An increase in cholesterol level is the sign of kidney damage\textsuperscript{43}. Carbamate pesticides are known to inhibit hepatic cytochrome P450 enzymes\textsuperscript{44}, which in turn leads to cholesterol accumulation\textsuperscript{25}. It has been reported that there was an increase in total cholesterol level in liver and kidney of mice treated with furadan\textsuperscript{45} and carbosulfan\textsuperscript{25,26}. The increase in cholesterol level in kidney in the study may be due to inhibitory action of pesticide on Cyt-p-450 or metabolic enzymes\textsuperscript{46,47} or due to high affinity binding\textsuperscript{48}, thereby affecting the enzymes which are essential for cholesterol break up causing deposition of cholesterol in the tissue.

The present findings revealed that, with increase in dose exposure of indoxacarb showed increase in LDH, ASAT and ALAT and decrease in the activity of SDH in the kidney of albino mice. Increased in high levels of activity LDH was reported in liver mice, orally treated with endosulfan and demonstrated by histochemical method\textsuperscript{49}. Similar observations were noted with effect of paraquat, methidation and copper sulphate pesticides \textsuperscript{50}. Mahagoub and Medany \textsuperscript{51} reported that chronic exposure of methomyl (17 mg/ kg body wt) for 2 months revealed significantly decreased SDH activity in rat testis. Similarly carbosulfan, methomyl and phosphomidon showed increase activity of LDH, ASAT and ALAT and decreased activity of SDH in mice\textsuperscript{26,27,28}. Increased permeability of cell and necrosis are usually characterized by rise in LDH activity\textsuperscript{52}. Similar reports are described in different animal species in response to heavy metals and pesticides \textsuperscript{53-56}. Moreover, several investigators have reported that the oxygen consumption and the activities of liver respiratory (SDH, MDH, NAD-Iso De) were decreased with an elevation of glucose-b-phosphate dehydrogenase, glyceraldehyde dehydrogenase and/or LDH activities in stressed animals. They suggested that the stressed animals are meeting its energy requirements through anaerobic oxidation\textsuperscript{57-62} additionally\textsuperscript{63} reported decrease in the activity of liver SDH suggests that anaerobic metabolism was favored over aerobic.
oxidation of glucose through Krebs cycle in order to mitigate the energy crisis for survival.

With increase in dose and prolong duration of exposure to indoxacarb caused decrease in the activity of Na⁺-K⁺ATPase, Mg⁺⁺ATPase and Ca⁺⁺ATPase in kidney of mice. It has been reported that elevation in LDH and CK and their isoenzymes in serum are due to loss of membrane permeability caused by depletion of ATP⁶⁴, ⁶⁵. Recently Ksheersagar and Kaliwal⁷⁵ reported that carbosulfan treated albino mice showed significantly decreased activity of ATPases and ACP in mouse liver and kidney, similar results were obtained due to intoxication of methomyl and phosphomidon⁷⁷, ⁷⁸. It has been reported that N – methyl carbamate inhibits ATPase system and alters calcium, magnesium homeostasis or energy related metabolic alterations⁶⁶-⁶⁸. Thus, the results obtained from the present observation indicate that, the indoxacarb caused inhibition of cell membrane Na⁺-K⁺ATPase, an important enzyme utilizing the energy from ATP hydrolysis for transport of several actions.

The present findings revealed that, with increase in dose and exposure of indoxacarb caused decrease in ACP and increase in AKP activity in kidney of mice. Similar results have been reported with different pesticides. Mancozeb treated rats produced significant enzymatic changes in the activity of ASAT, ALAT, AKP and LDH in liver, brain and kidney⁶⁹. Carbaryl led to marked increase in the activity of ASAT, ALAT, ACP, AKP and LDH in the serum of Clarias batrachus⁷⁰. Similarly Srivastava et. al.⁷¹ have reported that ASAT, ALAT, ACP and AKP were increased significantly in plasma, liver, kidney, lung, brain, heart, intestine and muscle of rat treated with dichlorovos and suggested that these results are due cellular damage or increased permeability of plasma membrane. 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 than induced by phenol intoxication ⁷². This result is confirmed by the marked rise of ASAT and ALAT activities that reported in the study. The enhanced activities of transaminases induced tissue proteolysis. This phenomenon is previously recorded for different fish species subjected to phenol⁷²-⁷⁴. The recorded elevated activities of transaminases in the muscle might have indicated a preliminary protein breakdown to obtain energy⁷². Thus, the results obtained
from the present observation indicate that, the indoxacarb caused increase in the AKP and decrease in ACP due to cellular damage or increased permeability of plasma membrane. The elevated activities of transaminases in the muscle might have indicated a preliminary protein breakdown to obtain energy to detoxify the indoxacarb.

ALP is membrane bound enzyme found at bile pole of hepatocytes and also found in pinocytic vesicle and Golgi complex. It is present on all cell membranes where active transport occurs and hydrolase and transphosphorylase in function. Inhibition of ALP reflects alterations in protein synthesis and uncoupling of oxidative phosphorylation. The decreasing ALP by stressors probably indicates an altered transport of phosphate and an inhibitory effect on the cell growth and proliferation. The inhibitors of ALP activities were demonstrated in animals exposed to different heavy metals, pesticides and sewage. It has been reported that methomyl treated rats showed significant decrease in level of Cytochrome P450, and significant increase in serum ASAT, ALAT and AKP. Thus in the present study elevation in ASAT, ALAT, ALP in the kidney suggests that indoxacarb causes deleterious effect on the kidney causing increased membrane permeability resulting in leakage of lysosomal enzymes.

In the present study, histopathological study of kidney of the mice revealed that increase in dose and durational exposure of methomyl caused loss of normal arrangement of cortical tubules and formation of vacuoles with flattened tubular cells. The glomerulus are small and atrophied, loosely attached to Bowman’s capsule. Vacuoles were found prominently due to loss of glomerulus. The present study suggests that the affected histoarchitecture of the kidney is due to susceptibility of kidney to methomyl induced toxicity. Similar results have also been observed in rats exposed to carbaryl. Subacute treatment of carbaryl showed increased vacuoles in the cells of proximal tubuli and hypertrophied glomerulus in rat kidneys. Cell inflammatory response, resulting from cell swelling, loss of plasma membrane integrity and leakage of cellular contents into the extra cellular space is certainly be necrotic condition of cells. Methyl carbamates are known to induce cell necrosis.

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EXPLANATION TO PHOTOMICROGRAPHS

Fig.1. T.S. of the kidney of the control mouse showing cortical tubules with normal arrangement. Thick epithelial cells with prominent glomerulus in Bowman’s capsule.

Fig.2. T.S. of the kidney of the mouse treated with 6 mg/ kg body weight/ day indoxacarb for 30 days showing loss of normal arrangement of cortical tubules. Tubular cells are hypertrophied.

Fig.3. T.S. of the kidney of the mouse treated with 12 mg/ kg body weight/ day indoxacarb for 30 days showing flattened tubular cells with vacuole formation. The cortical tubules lost normal arrangement.

Fig.4. T.S. of the kidney of the mouse treated with 18 mg/ kg body weight/ day indoxacarb for 30 days showing flattened tubular cells. The glomeruli are small and atrophied, loosely arranged in Bowman’s capsule.

Fig.5. T.S. of the kidney of the mouse treated with 24 mg/ kg body weight/ day indoxacarb for 30 days showing cortical tubules with enlarged lumen and flattened tubular cells. Glomeruli are atrophied and are loosely attached in Bowman’s capsule.

Photographs original exposure X 100
CT-Cortical tubules, G-Glomerulus, BC-Bowman’s capsule,
V-Vacuoles, TC-Tubular cells
Table 1—Effect of indoxacarb on kidney biochemical contents in albino mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Biochemical contents (µg / mg wet weight of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DNA</td>
</tr>
<tr>
<td>I.</td>
<td>Control</td>
<td>2.19 ± 0.04</td>
</tr>
<tr>
<td>II.</td>
<td>6 mg/kg/d</td>
<td>2.13 ± 0.03</td>
</tr>
<tr>
<td>III.</td>
<td>12 mg/kg/d</td>
<td>2.02 ± 0.04</td>
</tr>
<tr>
<td>IV.</td>
<td>18 mg/kg/d</td>
<td>1.80 ± 0.04*</td>
</tr>
<tr>
<td>V.</td>
<td>24 mg/kg/d</td>
<td>1.70 ± 0.05*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 10 animals

* Significant P 0.05 compared to control
Table 2—Effect of indoxacarb on kidney dehydrogenase, aminotransferase and phosphatase enzymes activity in albino mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Enzyme activity (μmoles / min / g tissue weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LDH^a</td>
</tr>
<tr>
<td>I.</td>
<td>Control</td>
<td>10.47 ± 0.45</td>
</tr>
<tr>
<td>II.</td>
<td>6 mg/kg/d</td>
<td>11.47 ± 0.46</td>
</tr>
<tr>
<td>III.</td>
<td>12 mg/kg/d</td>
<td>12.03 ± 0.48</td>
</tr>
<tr>
<td>IV.</td>
<td>18 mg/kg/d</td>
<td>12.88 ± 0.40*</td>
</tr>
<tr>
<td>V.</td>
<td>24 mg/kg/d</td>
<td>13.50 ± 0.55*</td>
</tr>
</tbody>
</table>

* Significant P < 0.05 compared to control.

Values are mean ± SEM of 10 animals.

a μmoles of pyruvate formed/min/g tissue
b μmoles formazon formed/min/g tissue.
c μmoles of inorganic phosphorus formed/min/g tissue.
d μmoles of p-nitrophenyl formed/min/g tissue.