Indoxacarb induces liver oxidative stress in Swiss Albino Mice

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ABSTRACT

Indoxacarb, (S)-methyl 7-chloro-2,5-dihydro-2-[(methoxycarbonyl) [4-(trifluoromethoxy) phenyl]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 liver 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 24 mg/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. Liver dissected out were freed from adherent tissue and weighed to nearest milligram. Liver oxidative stress byproducts of Lipid (Malonaldehyde) and protein (Protein carbonyl) contents were increased whereas GSH (Glutathione) and ascorbic acid contents were decreased in mice treated with 18 and 24 mg/kg/day indoxacarb. In mice treated with 18 and 24 mg/kg/day of indoxacarb showed increase in SOD (Superoxide dismutase), Catalase and GST (Gluthathione-s-transferase). However, there was no change in the oxidative stress byproducts and enzymes in the mice treated with 6 and 12 mg/kg/day indoxacarb. The results of the present study suggest that chronic exposure to indoxacarb insecticide has deleterious effect on liver.

Key Words: Liver, Indoxacarb, Oxidative stress byproducts, Oxidative stress enzymes.

INTRODUCTION

Oxidative stress is defined as a disruption of the prooxidant antioxidant balance in favor of the former, leading to potential damage [1]. It is reported that free radicals and other reactive species are derived either from normal essential metabolic processes or from external sources, such as exposure to x-rays, pesticides, cigarette smoking, air pollutants, industrial chemicals, etc [2]. It is a result of one of three factors i.e. an increase in reactive oxygen species (ROS), an impairment of antioxidant defense systems, or an insufficient capacity to repair oxidative damage. Damage induced by ROS includes alterations of cellular macromolecules such as membrane lipids, DNA, and proteins. The damage may alter all function through changes in intracellular calcium or intracellular pH, and eventually can lead to cell death [3,4].

There are reports that both acute and chronic carbafuran exposure results in perturbations in oxidative stress, several studies provide evidence that antioxidants may be used as biomarkers of exposure to environmental pollutants [5,6]. Moreover, many reported that various pesticides can induce oxidative stress in different tissues [7,8,9]. It has been reported that indoxacarb induces oxidative stress in testes and kidney [10,11] and induces changes in biochemical
constituents of liver, ovary and uterus [13,14] and also induces effects on estrous cycle and ovarian follicles in albino mice [12]. A number of previous studies have reported that lindane causes oxidative stress in the liver [15,16]. Several studies with liver, brain, and testes indicate that lindane causes oxidative stress [17-23]. Reactive oxygen species (ROS) generated by exogenous and endogenous factors, cellular metabolism and etc [24]. Dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) and polychlorinated biphenyls, which act as hepatic carcinogens 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 [25-30]. 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 [31,32]. Antioxidant such as ascorbic acid, vitamin E and GSH protect cells against oxidative DNA damage and play a important role in detoxification [33]. Therefore the present investigation was under taken to study the effect of indoxacarb on oxidative stress enzymes, antioxidants and oxidative stress byproducts of the liver 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 soyate 57.5%(w/w).

Structural Formula of Indoxacarb (C22H17ClF3N3O7)

Indoxacarb: (S)-methyl 7-chloro-2, 5-dihydro-2-[(methoxycarbonyl) [4-(trifluoromethoxy) phenyl] amino] carbonyl] indeno [1,2-e] [1,3,4] oxadiazine-4a (3H) carboxylate

Animals and treatment

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 (12h L: 12h 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 24mg/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 by (GSH) [34], ascorbic acid by [35], Thiobarbaturic acid (TBARS) [36], protein carbonyl [37], catalase by [38], superoxide dismutase (SOD) [39] and Glutathione-s-transferase (GST) [40].
Statistical Analysis
Statistical significance between control and experiment data were subjected to analysis of variance (ANOVA) together with Dunnett’s test (P<0.05).

RESULTS

Antioxidants, oxidative stress byproducts and oxidative stress enzymes
Results of the present study on antioxidants, oxidative stress byproducts and oxidative stress enzymes of the liver in mice revealed that the mice treated with 6 and 12 mg indoxacarb showed no significant change in the level of antioxidant, oxidative stress byproducts and oxidative stress enzymes. 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, protein carbonyl and showed significant increase in the activity of catalase, SOD, and glutathione s-transferase enzymes when compared with that of the control mice (table 1).

DISCUSSION

The present findings revealed that, an increase in the dose of indoxacarb showed decrease in the concentration of Glutathione (GSH). GSH plays a fundamental role in the antioxidant biology of mammals. GSH is widely distributed tripeptide and found mainly in the cell cytosol. 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 α-tocopherol, and serves as a substrate for GSH-related enzymes, e.g. glutathione peroxidase (GPx) and glutathione s-transferases [40]. It has been reported that in transgenic mice there is 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. The heightened paraquat sensitivity is also paradoxical in light of the general increase in GST expression in VP-hPX R mice. Paraquat is a quaternary nitrogen herbicide that causes toxic effects mainly via oxidative stress-induced mechanisms [41]. 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 [42] after carbamate exposure. 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) [43] who used a concentrate of technical dimethoate and pyrantel embonate. It has been revealed that 8-week oral administration of chlorpyrifos (at a dose of 13.5 mg/kg b.w.) to rats [44] observed a decreased level of hepatic GSH. They suggested that under oxidative stress, the content of GSH used by glutathione-dependent enzymes decreases. In the present study the reason for decreased GSH level in testes under the influence of indoxacarb treatment in mice may be due to the indoxacarb is a fluorinated compound prone to bind various antioxidants and anti-oxidation enzymes as it has been observed oxidative stress in the liver of mice exposed to different doses of F (NaF) [45-48]. Many of the metabolites of pesticides are also conjugated with glutathione, causing depletion of the glutathione reserve [49]. It is reported that the levels of lipid peroxidation were assessed by estimating TBARS and lipid peroxidation and the antioxidant levels were assessed by estimating the levels of GSH, SOD, CAT and GPx. Significant increases was observed in the levels of TBARS and hydroperoxide in CdCl2 treated rats [50].

The present findings revealed that, an increase in the dose of indoxacarb showed decrease in the concentration of ascorbic acid. Vitamin C-mediates quenching of mitochondrial ROS during normal and oxidative conditions correlate with the protective effect of vitamin C in inhibiting oxidative insults on the mitochondrial (mtDNA). 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) [51], 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. 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. 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) [51] 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. Łukaszewicz-Hussain and Moniuszko-Jakoniuk (2003) [52] 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 [53] after intoxication of rats with methylparathion. In the present study the reason for decreased concentration of ascorbic acid may be due to 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 [51]. The observed decrease in vitamin C concentration in the liver may also be 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 [54,55].

Table 1 Effect of indoxacarb on liver oxidative stress parameters in female 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>
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<tr>
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<tr>
<td>I</td>
<td>Control</td>
<td>GSH a</td>
<td>TBARS b</td>
<td>Catalase f</td>
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<tr>
<td></td>
<td></td>
<td>Ascorbic acid</td>
<td>Protein carbonyl c</td>
<td>SOD e</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GST f</td>
</tr>
<tr>
<td>II</td>
<td>1.75±0.18</td>
<td>430±40.0</td>
<td>14.00±4.7</td>
<td>0.045±0.001</td>
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<tr>
<td></td>
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<td></td>
<td>46.18±0.35</td>
</tr>
<tr>
<td>III</td>
<td>1.45±0.10</td>
<td>400±45.0</td>
<td>18.3±2.95</td>
<td>0.047±0.002</td>
</tr>
<tr>
<td>IV</td>
<td>1.38±0.08</td>
<td>375±30</td>
<td>22.2±1.95</td>
<td>0.049±0.003</td>
</tr>
<tr>
<td>V</td>
<td>1.20±0.04</td>
<td>320±25*</td>
<td>28.0±2.10*</td>
<td>0.059±0.002</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of 10 animals.

In the present investigation the increased levels of TBARS contents in liver of mice are found with higher dosage. Lipid peroxidation is a chain reaction between polyunsaturated fatty acids and ROS, and it produces lipid peroxides and hydrocarbon polymers that are both highly toxic to the cell. Malonyldialdehyde (MDA) is an end product of peroxidation of polyunsaturated fatty acids and related esters, and is, therefore, used as a marker of lipid peroxidation. Lipid peroxidation has been shown to increase in plasma and some tissues in Cypermethrin (CYP) and other insecticides toxicities [56-59]. The lipophilic characteristics of CYP indicate that the site of action is sodium channels in the neuronal membrane [60-62] 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. A number of previous studies have reported that lindane causes oxidative stress in the liver [15,18,23]. Hincal et al., (1995) [63] reported that 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. Findings suggested that the carbonfuran [CF] induces oxidative stress as evidenced by increased levels of lipid peroxidation, decreased GSH contents, and lowered activities of antioxidant enzymes. These results suggest that aqueous extract of M. charantia leads to a significant improvement [64]. In the present study the reason for increased TBARS level in liver under the influence of indoxacarb treatment in mice might caused due to the conjugation of indoxacarb or its metabolites to the polyunsaturated fatty acids or by production of ROS reacts with polyunsaturated fatty acids or accumulation of lipophilic compounds conjugated with the fatty acids.

In the present investigation the increased level of protein carbonyl in liver of mice are found with higher dosage of indoxacarb. Many environmental pollutants or chemicals exert their toxic effects by generating ROS [65,66] ROS are unstable free radical species in cells produced when oxidative stress occurs [65]. 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 [66]. Proteins are major targets for ROS and can scavenge 50-75% of ROS, as they are the major component of most biological systems [66]. Some ROS-induced protein modifications can result in unfolding or alteration of protein structure and functions [67]. 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 [68,69] this could be one of the reason increase in protein carbonyl. Generally, ROS may cause reversible and/or irreversible modifications on sensitive proteins [69]. Reversible modifications, usually at cysteine residues, may have a dual role of protection from irreversible damage and modulation of protein function [68]. Zarn et al., (2003) [70] have reported that protein carbonylation, but not thiol modification, in the mouse liver increased significantly during propiconazole induced oxidative stress. [71] 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 this could be another reason for the increase in protein carbonyl.

In the present investigation the increased activity of catalase and SOD in the liver of mice are found with higher dosage of indoxacarb Similar reports have been reported that SOD and CAT are the most important antioxidants in the body that play an important role in scavenging ROS. It has been reported that the increased activity of CAT, SOD and GPx was observed at the same time after intoxication with endosulfan. It has been reported that lindane-induced oxidative stress in the heart, liver and testes higher levels of SOD and catalase following adaptation might have protected the myocardium from more severe injury due to oxidative stress [49]. In the present study the reason for increased activity of catalase and SOD under the influence of indoxacarb treatment in mice might be due to the efficient clearance of ROS, however, requires the coordinate actions of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT) [72]. Investigations indicated that mammals 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, H$_2$O$_2$ at low concentration is destroyed by this enzyme [73-74].

In the present investigation the increased activity of Glutathione S-transferase in liver of mice are found with higher dosage of indoxacarb. 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 [75-77]. The heightened paraquat sensitivity is also paradoxical in light of the general increase in GST expression in VP-hPXR mice. 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 [78]. 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 [79,80]. Allen et al., (2006) [81] have reported that the percentage of LW (liver weight)/BW (Body weight) and activity in liver toxicity marker GST were increased significantly in the liver of propiconazole-treated mice. In the present study the reason for increased activity of Glutathione S-transferase under the influence of indoxacarb treatment in mice kidney due to ROS produced by the indoxacarb or to detoxify the pesticide or in order to eliminate the pesticide from the body by conjugation with the GSH to become more water soluble. It is revealed that concentration dependent increase in lipid peroxidation and alkaline phosphatase along with reduction in enzymatic and non-enzymatic antioxidants. Hence during aflatoxin infection and after exposure [82].

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