Effect of repeated oral administration of Butachlor on oxidative stress parameters and histopathological changes in wistar rats

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DOI: https://doi.org/10.22271/phyto.2022.v11.i1b.14321

Abstract

The experiment was conducted with wistar rats divided into two groups for a period of 28 days and was given through oral gavage. Group I served as control and was provided with water only for drinking purposes. Animals of Group II Animals of group 2 were administered Butachlor (Mancheter EC) @262 mg/kg B/W/P.O. Increase in lipid peroxidation and decrease in blood glutathione, glutathione peroxidase and transferase, catalase and superoxide dismutase in blood and tissues respectively revealed a clear picture of oxidative stress. Histopathologically, liver and kidney exhibited structural alterations as compared to control.

Keywords: herbicides, butachlor, oxidative stress, liver, kidney, histopathology, wistar rat

Introduction

The application of a wide variety of pesticides on crop plants is necessary to combat pests and vector borne diseases but this sporadic use has lead to significant consequences not only to public health but also to food quality resulting in an impact load on environment and hence the development of pest resistance. Butachlor is most commonly used herbicide which act by inhibiting the elongase that is responsible for elongation of very long chain fatty acids and geranyl pyrophosphate cyclization enzymes (Gotz and Boger., 2004). It also affects various other metabolic processes and redox homeostasis adversely, in addition to lipid biosynthesis (Agarwal et al., 2014) [2]. In India, nearly 6750 metric tons of butachlor are applied annually, as it was the first rice herbicide to be introduced (Verma et al., 2014). Release of butachlor to the environment is either by various waste streams from its production industry or by its release as pre-emergence herbicide.

Material and Methods

Adult Wistar rats of either sex were purchased from the Indian Institute of Integrative Medicine (Council of Scientific and Industrial Research Laboratory, Jammu) and maintained under standard experimental conditions. The experimental protocols were duly approved by the institutional ethical committee and experimental design was approved by the University Animal Ethical Committee. The animals were randomly divided into two groups with six rats in each group. The experiment was conducted for a period of 28 days and administration of toxicant was undertaken in morning between 8:00-9:00 A.M. The toxicant to the animals was given through oral gavage. Group I served as control and was provided with water only for drinking purposes. Animals of Group II Animals of group 2 were administered Butachlor (Mancheter EC) @262 mg/kg B.W/P.O.

Blood samples of about 2ml were collected from retro-orbital sinus of rats on 28th day using capillary-tubes in aliquots containing anticoagulant heparin strength @ 10 IU/ml of blood. Immediately after collection of blood, samples were centrifuged at 3000rpm for 15 minutes. Supernatant was discarded and normal saline was again added to the RBC on v/v basis, this process was repeated thrice. After final washing, phosphate buffer solution (pH 7.4) was taken as dilute to make 1 percent hemolysate and 33 percent hemolysate. For the estimation of catalase, superoxide-dismutase, glutathione-peroxidase and glutathione-S-transferase, 1 percent hemolysate was used and 33 percent hemolysate was used for estimation of lipid peroxidation. Rats were anesthetized with diethyl ether and eviscerated. 10 percent tissue homogenates of liver, kidney and lung tissues were prepared in 0.1 mol L−1 potassium phosphate buffer (pH 7.4).
Remaining tissue was stored in 10 percent formal saline solution for histopathological examination. Slides were prepared and stained with H&E as per method described by Luna (1968) [17]. For lipid peroxidation 1 ml of homogenate, 1 mL trichloroacetic acid was added. After vortexing, the mixture was centrifuged at 3000 rpm for 10 min. One milliliter of supernatant was mixed with 1 mL 2-thiobarbituric acid (TBA) and kept in boiling water bath for 10 minutes. This mixture was then cooled and diluted with 1 mL distilled water and its absorbance was read at 535 nm. (Shafiq-Ur-Rehman, 1984). For estimation of Superoxide dismutase, 1.5 mL tris-HCl buffer, 0.5 mL EDTA, and 1 mL pyrogallol solution were added in cuvette. The rate of auto-oxidation of pyrogallol was taken from the increase in absorbance at 420 nm, for 4 minutes after every 30 second lag.(Marklund and Marklund, 1974) [9]. For Catalase, 2 mL of phosphate buffer and 20 μL of homogenate was mixed well in cuvette. The reaction was started by the addition of 1 mL H2O2 and the decrease in absorbance was recorded at every 10 s interval for 1 min at 240 nm (Aebi, 1983) For Glutathione peroxidase estimation, 0.1 mL sample, 1 mL reduced glutathione (GSH), 1 mL sodium phosphate buffer and 0.5 mL sodium azide were added and the volume was made 4 mL with distilled water. After 5-minutes pre-incubation, 1 mL H2O2 (pre-warmed to 37 °C) was added to this mixture. Reduced glutathione concentration in protein-free filtrate was determined by mixing 2 mL of filtrate with 2 mL Na2HPO4 and 1 mL of 5–5- dithiobis-2-nitrobenzoic acid (DTNB) and the absorbance was recorded at 412 nm within 2 min after mixing. (Hafeman et al., 1974) [13] For Glutathione S-transferase 2.8 mL phosphate buffer, 0.1 mL GSH solution, and 20 μL of homogenate were mixed. The reaction was initiated by the addition of 0.1 mL 1- chloro-2, 4 dinitrobenzene (CDNB) prepared in 95 per cent ethyl alcohol. Increase in optical density at 340 nm was recorded for 3 min, after a lag of 30 s (Habig et al., 1974) [14].

**Table 1:** Effect of oral administration of Butachlor on non enzymatic parameters: Blood glutathione in Wistar rats (n=6).

| Group   | Treatment          | Blood Glutathione |
|---------|--------------------|-------------------|
| I       | Control            | 4.55±0.243        |
| II      | Butachlor@262 mg/kg| 3.77±0.119        |

**Lipid peroxidation/Malondialdehyde (MDA):** MDA is an end product of peroxidation of polysaturated fatty acids and related esters, and is, therefore used to estimate extent of lipid peroxidation (Sayed et al., 2003) [28]. Oxidative stress is associated with toxic reactive oxygen species and mammalian cells are induced with extensive antioxidant defense mechanism which counteracts the damaging effects of toxic reactive oxygen species (Halliwell and Guttridge, 1989) [11]. In present study, the levels in blood, hepatic tissue and renal tissue increased by 41.35 per cent, 35.16 per cent, and 34.32 per cent respectively. Similar results were observed by Farombi et al., 2018 [8] in fish. The data indicated that the reactive oxygen species may be associated with the metabolism of butachlor leading to peroxidation of membrane lipids of the respective organs. Previous studies have reported the induction of lipid peroxidation by other pesticides such as endosulfan (Pandey et al., 2001) [21] and cypermethrin (Uner et al., 2001) [26] in fish.

**Table 2:** Effect of oral administration of Butachlor on non enzymatic parameters: Malondialdehyde (MDA) in blood, hepatic tissue, renal tissue Wistar rats (n=6).

| Group   | Treatment          | Blood         | Hepatic tissue | Renal tissue |
|---------|--------------------|---------------|----------------|--------------|
| I       | Control            | 2.249±0.217   | 32.238±1.993   | 25.976±1.215 |
| II      | Butachlor@262 mg/kg| 3.835±0.445   | 49.710±3.150   | 39.557±2.461 |

**Superoxide dismutase (SOD):** SOD is first line of defence against the action of O2− and other reactive oxygen species (ROS). Superoxide radicals are produced in mitochondria and endoplasmic reticulum as a consequence of auto-oxidation of electron transport chain components. SOD is the major enzyme that protects against superoxide to hydrogen peroxide and oxygen (McCord and Fridovich, 1969) [20]. In present experiment SOD showed a statistically significant (P<0.05) decrease in blood, hepatic tissue and renal tissue by 24.73 per cent, 35.80 per cent, 40.38 per cent respectively. Decreased SOD activity is suggestive of excess free radical generation which impairs natural defense mechanism of this enzyme and are in consonance with the studies of Verma and Srivastava (2003) [29] in Chlorpyrifos intoxicated rats.
Glutathione peroxidase (GPx): It is a seleno-enzyme that protects biomembranes and other cellular components against oxidative damage. In present study, a statistically significant decrease in GPx was observed in blood, hepatic and renal tissue by 31.013%, 29.019%, 36.62% respectively. Present findings of decreased GPx levels are in agreement with studies of Verma and Srivastava, (2003) on chlorpyrifos treated rats. As per studies of Dong et al (2014), GPx exhibited a marked increase in liver and gills of fish administered with butachlor and slight increase in kidney tissues as compared to control.

Glutathione S-Transferase (GST): GST catalyze the conjugation of reduced glutathione via the sulphydryl group to electrophilic centers and this catalytic activity of combined glutathione with electrophiles helps in excretion of toxicant from cells and protects the tissues from the oxidative stress (Hayes and Paiford, 1995). In present study the level of GSH declined by 37.25 per cent, 16.53 per cent, and 51.16 per cent in blood, hepatic tissue and renal tissue respectively. These findings are in agreement with studies of Jackie et al (2011) and Verma and Srivastava (2003) on lead and chlorpyrifos respectively. Similarly, metal cadmium exposure increased ROS formation which in turn caused lipid peroxidation, DNA damage and oxidatively modified proteins which eventually leads to cellular dysfunction and necrotic cell death (Thevond, 2009).

Catalase (CAT): Catalase enzyme is found in all living organisms which catalyse the decomposition of hydrogen peroxide to water and oxygen (Chelikani et al., 2004). In present study the catalase activity declined in blood, hepatic tissue and renal tissue by 22.39 per cent, 30.54 per cent, and 33.36 per cent respectively. Our experimental design are in agreement with findings of Mannan et al., 2005 and Ahmet et al., 2005 in deltamethrin and cypermethrin treated rats respectively.

Histopathological studies

Histopathological alteration in hepatic tissue: The histomicrograph of liver section of control group I depicted normal histological appearance with different hepatic lobules containing central vein and hepatocytes, portal tracts with portal vein, hepatic artery, bile duct and lymphatics (Plate 1). Liver section of rats of group II administered with butachlor @262 mg/kg body weight showed hepatocytic degeneration and fatty change (Plate 3). Also, congestion and mild fibrosis of portal vein with degeneration of periporal hepatocytes was displayed (Plate 5). Histopathological observation of liver section of rat of group II (hepatocytic degeneration and fatty change) which is in agreement with findings of Black et al., 1994 with chlorfenapyr which functions as un-coupler of oxidative phosphorylation in mitochondria and Ahmadivand et al., 2014 with a similar study on the histopathological response of male trout subjected to butachlor.
Histopathological alteration in renal tissue: The histomicrograph of kidney section of control group I depicted normal histological appearance with usual appearance of glomeruli and tubules (Plate 6). Kidney section of rats of group II administered with butachlor@262 mg/kg b.wt. showed degeneration of tubules and fatty changes mildly (Plate 7). It is in agreement with findings of Ahmadivand et al., 2014 [1] with a similar study on the histopathological response of male trout subjected to butachlor. These findings may be attributed to the fact that kidneys are the main organs responsible for excretion and also due to residual accumulation of pesticide in the kidney tissue as mentioned by California EPA (2001a) [6]. Also infiltration of mononuclear inflammatory cells in many organs such as lungs, liver and kidneys explained leucopenia in rats of both treated groups as recorded by Davis (1981) [7].
Conclusion

The increasing incidence of suppression of immune system, chronic kidney problems, sterility among males and females, endocrine disorders, neurological and behavioral disorders, especially among children, have been attributed to pesticide poisoning. We designed this experiment to observe the oxidative stress induced by the pesticide (butachlor) by generation of free radical and histopathological changes induced in the major organ of detoxification and clearance (liver and kidney) with the aim to analyze the toxic effect an unprescribed pesticide can pose in the environment.

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