www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(11): 417-422

www.thepharmajournal.com
Received: 07-07-2022
Accepted: 13-09-2022

Uzma Farooq S
Division of Veterinary Pharmacology and Toxicology, Sher-e-Kashmir University of Agriculture Sciences and Technology Jammu, SKUAST-J, Jammu & Kashmir, India

Sharma A
Department of Veterinary Pathology, KCVAS, Amritsar, Punjab, India

Khajuria V
Department of Veterinary Parasitology, KCVAS, Amritsar, Punjab, India

Singh A
Department of Verterinary Pharmacology, KCVAS, Amritsar, Punjab, India

Kour H
Division of Veterinary Pharmacology and Toxicology, Sher-e-Kashmir University of Agriculture Sciences and Technology Jammu, SKUAST-J, Jammu & Kashmir, India

Corresponding Author:
Uzma Farooq S
Division of Veterinary Pharmacology and Toxicology, Sher-e-Kashmir University of Agriculture Sciences and Technology Jammu, SKUAST-J, Jammu & Kashmir, India

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

Uzma Farooq S, Sharma A, Khajuria V, Singh A and Kour H

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 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. Histopathologic ally, 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) [9]. 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) [30]. 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 diluent 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 33percent 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) [22]. 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) [19]. 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) [3]. 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) reagent 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].

Histopathological technique

Representative tissue pieces approximately 0.5 cm each were collected amd immediately fixed in 10% neutral buffered formalin (NBF) for 48-72 hours with 2-3 changes of formalin. After fixation in 10% NBF the tissue samples were trimmed to 1.5 mm thickness and given overnight washing under running tap water. The tissue samples were than dehydrated by passing through ascending grades of ethyl alcohol, cleared in xylene and embedded with paraffin wax (melting point 58 ºC) for block making. The sections were cut at 4-5µm thickness and stained by H&E stain as per standard procedure (Luna, 1968) [17].

Results and Discussion

Increase in lipid peroxidation and decrease in blood glutathione, glutathione peroxidase and transferase, catalase and superoxide dismutase in blood and tissues depicted Histopathologic ally, liver and kidney exhibited structural alterations as compared to control.

Oxidative parameters

Blood glutathione (GSH)

Glutathione (GSH) is an important naturally occurring antioxidant, which prevents free radical damage and helps detoxification by conjugating with xenobiotics. In addition, GSH is pivotal to the cellular antioxidant defenses by acting as an essential cofactor for antioxidant enzymes including glutathione perioxidase (GPx) and Glutathione-S-transferase (GST) (Hayes *et al*., 2005) [33]. In present study, a non-significant decline by 17.14 per cent was observed. Decrease in glutathione results in the impairment of mechanisms of metabolic detoxification (Verma and Srivastava, 2001) [27]. Similar results were found by Sharma *et al.* (2005) [23] following sub chronic exposure to the insecticide dimethioate (6 and 30 mg/kg) that resulted in decreased glutathione levels in both liver and brain tissues of male Wistar rats. Further studies of Goel *et al* (2005) [34] and Verma *et al* (2001) [27] showed depletion of GSH in chlorpyrifos-intoxicated animals.

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^{b}±0.243
II	Butachlor@262 mg/kg	3.77^{b}±0.119

Lipid peroxidation / malondialdehyde (MDA)

MDA is an end product of peroxidation of polyunsaturated fatty acids and related esters, and is, therefore used to estimate extent of lipid peroxidation (Sayeed *et al*., 2003) [24]. 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 Gutterridge, 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^{a}±0.217	32.238^{a}±1.993	25.976^{a}±1.215
II	Butachlor @ 262 mg / kg	3.835^{bc}±0.445	49.710^{bc}±3.150	39.557^{b}±2.461

Superoxide dismutase (SOD): SOD is first line of defence against the action of O_2^- 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) [27] in Chlorpyrifos exposed rats.

Table 3: Effect of oral administration of Butachlor on enzymatic parameters: Superoxide dismutase (SOD) in blood, hepatic tissue, renal tissue Wistar rats (n = 6).

Grp	Treatment	Blood	Hepatic tissue	Renal tissue
I	Control	$51.00^{d}\pm3.73$	$350.391^{e}\pm22.104$	$338.672^{e}\pm31.454$
II	Butachlor @ 262 mg / kg	$38.39^{abc}\pm2.99$	$224.950^{bc}\pm17.600$	$201.904^{bc}\pm11.256$

Glutathione peroxidase (GPx)

It is a seleno-enzyme that protects bio membranes 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) [27] on chlorpyrifos treated rats. As per studies of Dong *et al* (2014) [31], GPx exhibited a marked increase in liver and gills of fish administered with butachlor and slight increase in kidney tissues as compared to control.

Table 4: Effect of oral administration of Butachlor on enzymatic parameters: Glutathione peroxidase (GPx) in blood, hepatic tissue, renal tissue Wistar rats (n = 6).

Group	Treatment	Blood	Hepatic tissue	Renal tissue
I	Control	$8.232^{f}\pm0.553$	$52.041^{d}\pm4.197$	$41.546^{d}\pm4.952$
II	Butachlor @ 262 mg / kg	$5.679^{cd}\pm0.329$	$36.939^{c}\pm3.365$	$26.330^{b}\pm3.133$

Glutathione-S-Transferase (GST)

GST catalyze the conjugation of reduced glutathione via the sulfhydryl 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) [12]. 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) [15] and Verma and Srivastava (2003) [27] 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) [25].

Table 5: Effect of oral administration of Butachlor on enzymatic parameters: Glutathione-S-transferase (GST) in blood, hepatic tissue, renal tissue Wistar rats (n = 6).

Group	Treatment	Blood	Hepatic tissue	Renal tissue
I	Control	$0.51^{d}\pm0.02$	$180.32^{d}\pm10.4$	$90.975^{d}\pm7.21$
II	Butachlor @ 262 mg / kg	$0.32^{cd}\pm0.03$	$150.5^{cd}\pm6.8$	$44.425^{c}\pm2.14$

Catalase (CAT)

Catalase enzyme is found in all living organisms which catalyses the decomposition of hydrogen peroxide to water and oxygen (Chelikani *et al*., 2004) [32]. 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 [18] and Ahmet *et al*., 2005 [4] in deltamethrin and cypermethrin treated rats respectively.

Table 6: Effect of oral administration of Butachlor on enzymatic parameters: Catalase (CAT) in blood, hepatic tissue, renal tissue Wistar rats (n = 6).

Group	Treatment	Blood	Hepatic tissue	Renal tissue
I	Control	$55.72^{d}\pm3.64$	$3264.93^{e}\pm237.86$	$2637.32^{e}\pm190.28$
II	Butachlor @ 262 mg / kg	$43.24^{bc}\pm3.75$	$2267.17^{c}\pm172.51$	$1757.47^{cd}\pm129.26$

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 periportal 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 Ahmadiv and *et al*., 2014 [1] with a similar study on the histopathological response of male trout subjected to butachlor.

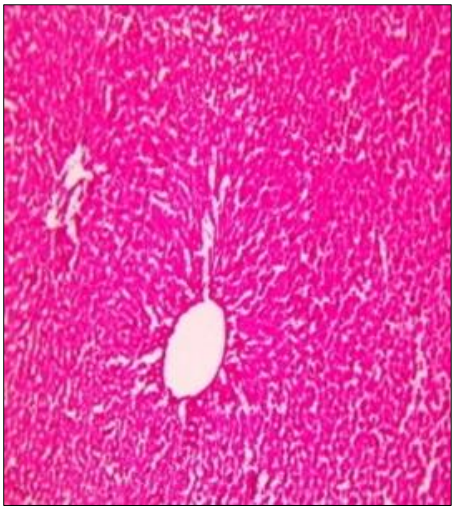

Plate 1: Liver: Group I: Showing Normal Liver H&E×100

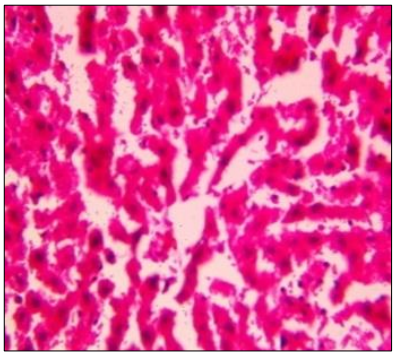

Plate 2: Showing higher magnification of plate 1 H&E ×400

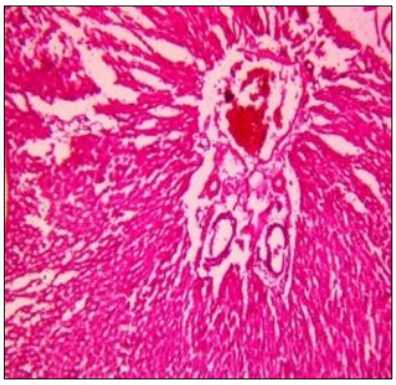

Plate 3: Liver: Showing hepatocytic degeneration and fatty changes H&E× 100

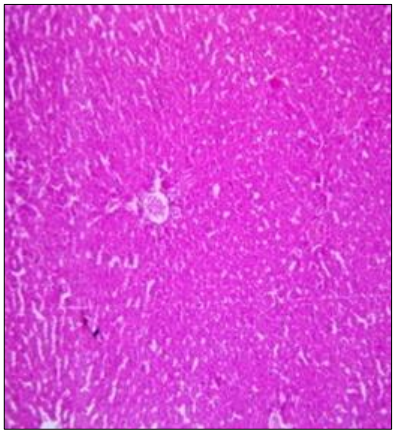

Plate 4: Showing higher magnification of H&E×400

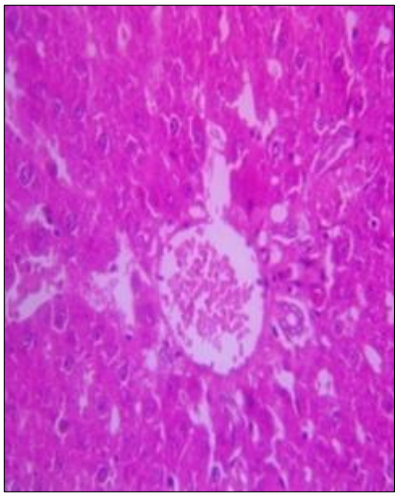

Plate 5: Portal area showing congested portal Vein and mild fibrosis and periportal hepatocytic degeneration H&E× 100

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). 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].

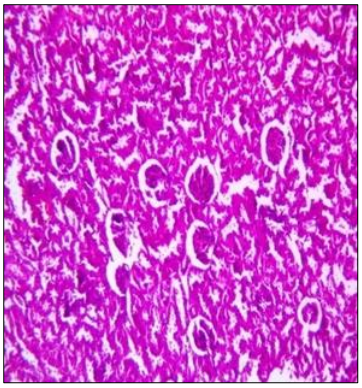

Plate 6: Kidney: Group I: Normal kidney H&E ×100

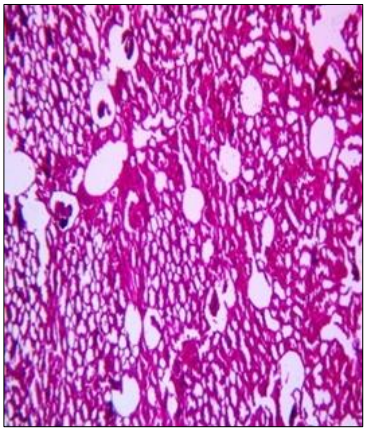

Plate 7: Kidney: group II: Showing cystic dilatation of glomeruli with atrophy of glomerular tuft. H&E ×100

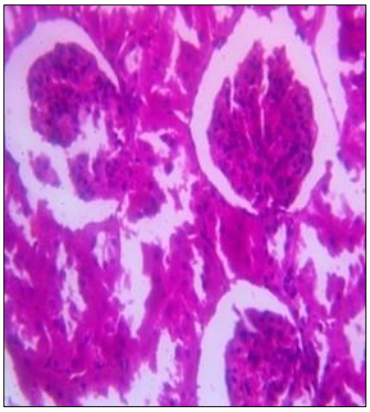

Plate 8: Showing higher magnification of H&E×400

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.

References

1. Ahmadivand S, Farahmand H, Mirvghefi AR, Eagderi S, Shokrpoor S, Holasoo R. Histopathological and haematological response of male rainbow trout subjected to butachlor. Veterinary medicine. 2014;59(9):433-439.
2. Agarwal C, Sen S, Singh S, Rai S, Singh PK, Singh VK, *et al*. Comparative proteomics reveals association of early accumulated proteins in conferring butachlor tolerance in three N2-fixing Anabaena species. Journal of Proteomics. 2014 Jan;96:271-290.
3. Aebi HE, (3rdeds.). Catalase in methods of enzymatic analysis. HU Bergmeyer, Weinheim: Verlagchemie, Academic press. New York; c1983. p. 276-286.
4. Ahmet A, Seval Y, Izzet K, Ibrahim P, Bulent T. The effects of Vitamin E and selenium on cypermethrin induced oxidative stress in rats. Turkish Journal of Veterinary & Animal Sciences. 2005;29(2):385-391.
5. Black BC, Hollingworth RM, Ahammadsahib KI, Kukel CD, Donovan S. Insecticidal action and mitochondrial uncoupling activity of AC-303, 630 and related halogenated pyrroles. Pesticide Biochemistry and Physiology. 1994 Oct 1;50(2)::115-128.
6. California EPA. A one-year dietary neurotoxicity study with AC 303, 630 in rats. Department of Pesticide Regulation, Medical Toxicology Branch; c2001a. Aug 24.
7. Davis DM. Textbook of adverse drug reaction. 2nd Ed. Oxford University Press, New York; c1981.
8. Farombi EO, Ajimoko YR, Adelowo OA. Effect of Butachlor on antioxidant enzyme status and lipid peroxidation in fresh water African Catfish, (*Clarias gariepinus*). International Journal of Environmental Research and Public Health Journal. 2008 Dec;5(5):423-427.
9. Go¨tz T and Bo¨ger P. The very-long-chain fatty acid synthase is inhibited by chloroacetamides. Zeitschrift für Naturforschung C. 2004 Aug 1;59(7-8):549-553.
10. Goel A, Dani V, Dhawan DK. Protective effects of zinc in Chlorpyriphos induced hepatotoxicity: a biochemical and trace elemental study, Biology of Trace element Research. 2000 May;74(2):171-183.
11. Halliwell B, Gutterridge JMC. (eds.). Free radicals in biology and medicine, Clarendon press, Oxford; c1989. p. 188-276.
12. Hayes JD, Pariform DJ. The glutathione- S- transferase supergene family. Regulation of GST and the contribution of the isoenzymes to cancer chemo protection and drug resistance. Critical Reviews in Biochemistry and Molecular Biology. 1995 Jan 1;30(6):445-600.
13. Hafeman DG, Sunde RA. Hoekstra WB. Effect of dietary selenium on erythrocyte and Glutathione peroxidise in rats. Journal of Nutrition. 1974;104:580-587.
14. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-Transferase. The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry. 1974 Nov 25;249(22):7130-7139.
15. Jackie T, Nagaraja H, Srikumar C. Antioxidant effects of Etlingeraelatior flower extract against lead acetate induced perturbations in free radical scavenging enzymes and lipid peroxidation in rats. BMC Research Notes. 2011 Dec;4(1):01-08.
16. Kanan K, Tanabe S, Ramesh A, Subramanian A, Tatsukawa R. Persistent organo chlorine residues in food stuffs from India and their implications on human dietary exposure. Journal of Agricultural and Food Chemistry. 1992 Mar;40(3):518-524.
17. Luna A and Lee G. (Eds.). Manual of histological staining methods of the armed forces institute of pathology, In: Luna, LG (3rd Eds.), American Registry of pathology; McGraw-Hill Book Company, New York, Toronto, London and Sydney; c1968. p. 258.
18. Manna SM, Bhattacharya D, Mandal TK, Das S. Repeated dose toxicity of deltamethrin in rats. Indian Journal of pharmacology. 2005 May 1;37(3):160-164.
19. Marklund S, Marklund M. Involvement of superoxide anion radical in auto-oxidation of pyrogallol and a convenient assay of superoxide dismutase. European Journal of Biochemistry. 1974;47:469-474.
20. McCord JM, Fridovich I. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprien). Journal of Biological Chemistry. 1969 Nov 25;244(22):6049-6055.
21. Pandey S, Ahmad I, Parvez S, Bin-Hafeez B, Haque R, Raisuddin S. Effect of endosulfan on antioxidants of freshwater fish *Channa punctatus* Bloch: 1. Protection against lipid peroxidation in liver by copper pre-exposure. Archives of Environmental Contamination and Toxicology. 2001 Oct;41(3):345-252.
22. Shafiq-ur-Rehman. Lead induced regional lipid peroxidation in brain. Toxicology letters. 1984 Jan 1;21(3):333-337.
23. Sharma Y, Bashir S, Irshad M, Nag TC, Dogra TD. Dimethoate – induced effects on antioxidants status of liver and brain of rats following sub chronic exposure. Toxicology. 2005 Nov 15;215(3):173-181.
24. Sayeed I, Parvez S, Pandey S, Bin- Hafeez B, Haque R Raisuddin. Oxidative Stress Biomarkers of Exposure to deltamethrin in fresh water fish, *Channa punctatus* Blonch. Ecotoxicology Environmental Safety. 2003 Oct 1;56(2):295-301.
25. Thevond F. Cadmium and cellular signalling cascades: to be or not to be. Toxicology and Applied Pharmacology. 2009 Aug 1;238(3):221-239.
26. Uner N, Oruc EO, Canli M, Sevgiler Y. Effects of cypermethrin on antioxidant enzyme activities and lipid peroxidation in liver and kidney of fresh water fish, *Oreochromis niloticus* and *Cyprinus caprio* (L.). Bull Environmental Contamination and Toxicology. 2001 Nov 1;67(5):657-664.
27. Verma RS, Srivastava N. Chlorpyrifos induced alterations in levels of thiobarbituric acid reactive substances and glutathione in rat brain. Indian Journal of Experimental Biology. 2001;39:174-178.
28. Verma RS, Srivastava N. Effects of Chlorpyrifos on thiobarbituric acid reactive substances, scavenging

enzymes and glutathione in rat's tissue. Indian Journal of Biochemistry and Biophysics. 2003;40:423-428.
29. Verma JP, Jaiswal DK, Sagar R. Pesticide relevance and their microbial degradation. Environmental Science and biotechnology Bio-technol; c2005.
30. Verma A, Cairns JA, Mitchell LB, Macle L, Stiell IG, Gladstone D, *et al*. Focused update of the Canadian Cardiovascular Society Guidelines for the management of atrial fibrillation. Canadian Journal of Cardiology. 2014 Oct 1;30(10):1114-30.
31. Dong Y, Yan Y, Zhang S, Li J. Wood/polymer nanocomposites prepared by impregnation with furfuryl alcohol and nano-SiO2. Bio Resources. 2014 Aug 18;9(4):6028-40.
32. Chelikani P, Fita I, Loewen PC. Diversity of structures and properties among catalases. Cellular and Molecular Life Sciences CMLS. 2004 Jan;61(2):192-208. DOI: 10. 1007/s11157-014-9341-47
33. Hayes AF, Glynn CJ, Shanahan J. Validating the willingness to self-censor scale: Individual differences in the effect of the climate of opinion on opinion expression. International Journal of Public Opinion Research. 2005 Dec 1;17(4):443-55.
34. Queipo NV, Haftka RT, Shyy W, Goel T, Vaidyanathan R, Tucker PK. Surrogate-based analysis and optimization. Progress in aerospace sciences. 2005 Jan 1;41(1):1-28.