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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

Nabi B
Division of Veterinary Medicine,
Sher-e-Kashmir University of
Agriculture Sciences and
Technology Jammu, SKUAST-J,
Jammu & Kashmir, India

Mahajan D
Department of Livestock
Production and Management,
KCVAS, Amritsar, Punjab,
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 and lead alone and in combination on oxidative stress and histopathology in wistar rats

Uzma Farooq S, Sharma A, Khajuria V, Nabi B and Mahajan D

Abstract

The experiment was carried out to evaluate the rising concern over additive effect of one chemical with another chemical which have raised a lot of health concern in general population. In the experiment, adult wistar rats of either sex were divided into 4 groups and study was conducted for a period of 28 days. Group I served as control and was provided with water only for drinking purposes. Animals of group II were administered butachlor @ 262 mg / kg B.W/P.O. Group III received administered lead acetate @ 265 ppm orally while animals of group IV received combination of butachlor @ 262 mg / kg and lead acetate @ 265 ppm. Oxidative stress was revealed by significant increase in lipid peroxidation and decrease in blood glutathione, glutathione peroxidase and transferase, catalase and superoxide dismutase in blood and tissues respectively. Histopathologic ally, liver and kidney exhibited structural alterations as compared to control. The study concluded butachlor and lead produced more deleterious systemic effect in combination than alone (additive toxic effect).

Keywords: Herbicides, butachlor, lead acetate, oxidative stress, histopathology, wistar rats

1. Introduction

The sporadic use of herbicide to control pests has lead to significant consequences not only to public health but also to food quality resulting in an impact load on environment. Butachlor is a widely recommended herbicide for rice cultivation. Release of butachlor to the environment is either by various waste streams from its production industry or by its release as pre-emergence herbicide. The Eco toxicological studies suggest that butachlor may be harmful to aquatic invertebrates (Valloton *et al.*, 2009) [38] and also a possible carcinogen in animals and humans (Geng *et al.*, 2005) [15]. The usage of effluents for agriculture purposes leading to contamination of food with heavy metals has been reported frequently. In recent years, lead has become a regulatory concern and subject of much interest among pharmacologist, environmental scientist and clinicians because of its widespread distribution in environment and its pharmacological behaviour to remain bound to mammalian tissues for a long duration (Freeman, 1970) [14]. Increased lead in environment leads to contamination of soil and fodder and continuous ingestion of such contaminated fodder results in chronic lead poisoning in animals. In India, several reports documenting lead poisoning in livestock in various parts of the country are now on record as compared to very few before 1980 (Dogra *et al.*, 1996) [11]. It is one of the most toxic metals known due to its wide ranging effects of multiple body system.

2. 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 (Manchester 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 3000 rpm 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) [23]. 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) [31]. 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) [26]. 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) [2]. 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) [20] 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) [21].

2.1 Histopathological Technique: Representative tissue pieces approximately 0.5 cm each were collected and 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 then dehydrated by passing through ascending grades of ethyl alcohol, cleared in xylene and embedded with paraffin wax (melting point 58 °C) for block making. The sections were cut at 4-5µm thickness and stained by H&E stain as per standard procedure (Luna, 1968) [23].

3. Results and Discussion

Oxidative stress as revealed by alteration in different biomarkers like increase in lipid peroxidation and decrease in blood glutathione (GSH), glutathione peroxidase and transferase (GPx & GST), catalase (CAT) and superoxide dismutase (SOD) in blood and tissues respectively was observed. Histopathologic ally, liver and kidney exhibited structural alterations as compared to control.

3.1. Oxidative Parameters

3.1.1. Blood glutathione (GSH): Glutathione (GSH) is an important naturally occurring antioxidant, which prevents free radical damage and helps detoxification by conjugating with xenobiotics. Under oxidative stress, GSH is depleted by GSH related enzymes to detoxify the peroxides produced due to increased lipid peroxidation (Cathcart, 1985) [42]. In present study, a non-significant decline by 17.14 per cent and 14.72 per cent was observed in group II and group III respectively and significantly 54.06 per cent decline was observed in group IV which received a combination of butachlor and lead respectively. Decrease in glutathione results in the impairment of mechanisms of metabolic detoxification (Verma and Srivastava, 2001) [39]. Similar results were found by Sharma *et al* (2009) [34] 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) [43] showed depletion of GSH in chlorpyrifos-intoxicated animals. The decline in GSH level in blood and brain of lead acetate @ 2000 ppm exposed rats were reported earlier by Tandon *et al.*, 2002 [35]. In addition, many of the metabolites of pesticides are also conjugated with glutathione, causing depletion of the glutathione reserve (Ananya *et al.*, 2005) [4] [Table 01].

Table 1: Effect of oral administration of butachlor and lead, alone and in combination 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
III	Lead @ 265 ppm	3.88 ^b ±0.09
IV	Butachlor @262 mg/kg + Lead@265 ppm	2.09 ^a ±0.13

3.1.2. Lipid peroxidation / malondialdehyde (MDA): Malondialdehyde (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) [32]. It acts as one of the markers of lipid peroxidation of lipid membrane damage by reactive oxygen species (ROS) (Linden *et al* 2008). 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 Gutteridge, 1989) [18]. In present study, the group II (butachlor) levels in blood, hepatic tissue and renal tissue increased by 41.35 per cent, 35.16 per cent, and 34.32 per cent respectively. In group III (lead) levels increased by 25.65 per cent, 32.57 per cent, and 33.26 per cent in blood, hepatic tissue and renal tissue respectively. In combination group IV (Butachlor and lead) levels increased by 54.37 per cent, 44.17 per cent and 47.96 per cent respectively in blood, hepatic tissue and renal tissue. Similar results were observed by Farombi *et al.*, 2018 [13] 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) [29] and cypermethrin (Uner *et al.*, 2001) [37] in fish. The rats exposed to lead acetate exhibited increase in MDA level in RBC, liver and kidney tissue. The finding of current study is in agreement with Tandon *et al* (2002) [35] who observed increased MDA levels in erythrocytes and

decreased GSH levels in blood and brain of rats receiving 2000 ppm of lead acetate. Patra and Swarup, (2001) [30] reported significantly higher lipid peroxide levels in liver, kidney and brain in lead exposed rats. The observed results are also in agreement with Gurer *et al* (1999) [17] who reported increased concentrations of MDA in liver, brain and kidney among lead acetate treated rats. Oteiza and Bechara (1993) [28] have also demonstrated that lead induced lipid peroxidation, might be as result of enhanced levels of L-amino levulinic acid that accumulates after lead exposure [Table 02].

Table 2: Effect of oral administration of butachlor and lead, alone and in combination on non-enzymatic parameters: Malondialdehyde (MDA) in blood, hepatic tissue, renal tissue in 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
III	Lead @ 265 ppm	3.025 ^{ab} ±0.373	47.807 ^{bc} ±2.695	38.922 ^b ±2.793
IV	Butachlor @ 262 mg / kg + Lead @ 265 ppm	4.929 ^c ±0.381	57.749 ^{cd} ±3.121	49.922 ^c ±3.287

3.1.3. 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. These superoxide free radicals are generated during monovalent reduction of oxygen and are toxic to biological systems. SOD is the major enzyme that protects against superoxide to hydrogen peroxide and oxygen (McCord and Fridovich, 1969) [27]. Decreased SOD activity in the present study is suggestive of excess free radical generation which impairs natural defense mechanism of this enzyme. In present experiment, group II (butachlor) 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. In group III treated with lead, decrease in the levels were 20.49 per cent, 77.58 per cent, 25.10 per cent in blood, hepatic tissue and renal tissue respectively. In group IV treated with butachlor and lead, a significant decrease with 46.96 per cent, 45.24 per cent, and 40.43 per cent in blood, hepatic tissue and renal tissue was observed respectively. These finding are in consonance with the studies of Verma and Srivastava (2003) [39] in chlorpyrifos exposed rats. This observation support the hypothesis that SOD activity is stimulated by an increased superoxide radical generation associated with the decline of SOD and glutathione peroxidase (Allen and Balin, 1989) [5] generated by the inhibitory action of lead acetate [Table 03].

Table 3: Effect of oral administration of butachlor and lead, alone and in combination on enzymatic parameters: Superoxide dismutase (SOD) in blood, hepatic tissue, renal tissue in wistar rats (n = 6).

Group	Treatment	Blood	Hepatic tissue	Renal tissue
I	Control	51.00 ^d ±3.73	350.391 ^e ±22.104	338.672 ^e ±31.454
II	Butachlor @ 262 mg / kg	38.39 ^{abc} ±2.99	224.950 ^{bc} ±17.600	201.904 ^{bc} ±11.256
III	Lead @ 265 ppm	40.55 ^{abc} ±5.57	271.858 ^{cd} ±20.182	253.643 ^{cd} ±32.517
IV	Butachlor @ 262 mg / kg + Lead @ 265 ppm	27.05 ^a ±5.18	191.860 ^b ±22.692	201.744 ^{bc} ±13.817

3.1.4. Glutathione peroxidase (GPx): It is a seleno-enzyme that protects bio-membranes and other cellular components against oxidative damage. The enzyme catalyzes the reduction of a variety of organic hydro peroxidases and lipid hydro peroxidases using glutathione as the reducing equivalent (Lui and Luo, 2003) [25]. In present study, a statistically significant decrease in GPx was observed in group II (butachlor) in blood, hepatic and renal tissue by 31.013 per cent, 29.019 per cent, 36.62 per cent respectively, in group III (lead) the levels declined by 25.93 per cent, 24.57 per cent, 11.62 per cent in blood, hepatic and renal respectively. In group IV (butachlor with lead) the levels decreased by 51.03 per cent, 47.91per

cent, 43.06 per cent respectively in blood, hepatic tissue and renal tissue. Present findings of decreased GPx levels are in agreement with studies of Verma and Srivastava, (2003) [39] on chlorpyrifos treated rats. As per studies of Dong *et al* (2015) [12], GPx exhibited a marked increase in liver and gills of fish administered with butachlor and slight increase in kidney tissues as compared to control. Our results are also in accordance to the findings of Shagirtha *et al.*, 2011 [33] who reported a significant decrease in the activities of enzymatic antioxidants like GSH, GPx, GST, G-6-P-D in kidney of Cd intoxicated rats given Cd @ 5 mg / kg bwt / day for 4 weeks [Table 04].

Table 4: Effect of oral administration of butachlor and lead, alone and in combination on enzymatic parameters: Glutathione peroxidase (GPx) in blood, hepatic tissue, renal tissue in wistar rats (n = 6).

Group	Treatment	Blood	Hepatic tissue	Renal tissue
I	Control	8.232 ^f ±0.553	52.041 ^d ±4.197	41.546 ^d ±4.952
II	Butachlor @ 262 mg / kg	5.679 ^{cd} ±0.329	36.939 ^c ±3.365	26.330 ^b ±3.133
III	Lead @ 265 ppm	6.097 ^{de} ±0.430	39.254 ^c ±2.715	36.713 ^{cd} ±4.933
IV	Butachlor @ 262 mg / kg + Lead @ 265 ppm	4.030 ^{ab} ±0.436	27.104 ^b ±2.748	23.650 ^b ±2.505

3.1.5. Glutathione -S-Transferase (GST): Glutathione -S-Transferase (GST) is a major group of enzyme that constitutes about 10 per cent of cytosolic protein in some mammalian organs. GST catalyze the conjugation of reduced glutathione via the sulfhydryl group to electrophilic centers on a wide variety of substances. In addition GST also binds with varying affinities to variety of hydrophobic compounds such as polycyclic aromatic hydrocarbons and other xenobiotics such as pyrethroids (Singh *et al.*, 2009) [34]. This activity is useful in the detoxification of endogenous compounds such as peroxidized lipids as well as metabolite of xenobiotics. 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) [19]. In present study, the group II (butachlor) level of GST

declined by 37.25 per cent, 16.53 per cent, and 51.16 per cent in blood, hepatic tissue and renal tissue respectively, In group III (lead) level of GST declined by 11.76 per cent, 6.11 per cent, 42.22 per cent in blood, hepatic tissue and renal tissue respectively. In Group IV (combination of butachlor and lead), the levels declined drastically by 50.98 per cent, 41.66 per cent, 61.39 per cent in blood, hepatic tissue and renal tissue respectively. These findings are in agreement with studies of Jackie *et al* (2011) [22] and Verma and Srivastava (2003) [39] 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) [36] [Table 05].

Table 5: Effect of oral administration of butachlor and lead, alone and in combination on enzymatic parameters: Glutathione-S-transferase (GST) in blood, hepatic tissue, renal tissue in wistar rats (n = 6)

Group	Treatment	Blood	Hepatic tissue	Renal tissue
I	Control	0.51 ^d ±0.02	180.32 ^d ±10.4	90.975 ^d ±7.21
II	Butachlor @ 262 mg / kg	0.32 ^{cd} ±0.03	150.5 ^{cd} ±6.8	44.425 ^c ±2.14
III	Lead @ 265 ppm	0.45 ^{cd} ±0.03	169.3 ^{cd} ±8.2	52.025 ^{cd} ±3.73
IV	Butachlor @ 262 mg / kg + Lead @ 265 ppm	0.25 ^{bcd} ±0.02	105.2 ^b ±5.4	35.125 ^b ±2.51

3.1.6. Catalase (CAT): Catalase is a common enzyme found in all living organisms which are exposed to oxygen where its function is to catalyse the decomposition of hydrogen peroxide to water and oxygen (Chelikanin *et al.*, 2004) [9]. Decreased catalase activity suggests lesser activity of superoxide dismutase which normally produces less effective H₂O₂ (Zakaryan *et al.*, 2002) [41]. In present study the catalase activity in group II (treated with butachlor) declined in blood, hepatic tissue and renal tissue by 22.39 per cent, 30.54 per

cent, and 33.36 per cent respectively, in group III (treated with lead) declined by 20.10 per cent, 22.61 per cent, 28.57 per cent respectively in blood, hepatic tissue and renal tissue. In the combination group (butachlor with lead), the catalase activity declined with 32.03 per cent, 50.83 per cent, 43.88 per cent in blood, hepatic tissue and renal tissue respectively. Our experimental design is in agreement with findings of Ahmet *et al.*, 2005 [3] in deltamethrin and cypermethrin treated rats respectively [Table 06].

Table 6: Effect of oral administration of butachlor and lead, alone and in combination on enzymatic parameters: Catalase (CAT) in blood, hepatic tissue, renal tissue in wistar rats (n = 6).

Group	Treatment	Blood	Hepatic tissue	Renal tissue
I	Control	55.72 ^d ±3.64	3264.93 ^e ±237.86	2637.32 ^e ±190.28
II	Butachlor @ 262 mg / kg	43.24 ^{bc} ±3.75	2267.17 ^e ±172.51	1757.47 ^{cd} ±129.26
III	Lead @ 265 ppm	44.52 ^{bc} ±2.84	2526.50 ^{cd} ±169.97	1883.59 ^d ±102.56
IV	Butachlor @ 262 mg / kg + Lead @ 265 ppm	37.87 ^b ±3.69	1605.23 ^b ±147.44	1479.85 ^d ±102.20

3.2. Histopathological studies

3.2.1. 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, lined in cord like fashion around it, clearly delineated portal tracts with portal vein, hepatic artery, bile duct and lymphatics. Liver section of rats of group II administered with butachlor @ 262 mg / kg body weight depicts hepatocytic degeneration and fatty change (Plate 1). Congestion and mild fibrosis of portal vein with degeneration of periportal hepatocytes was also prominent (Plate 2&3). Liver section of rats of group III administered with lead acetate @ 265 ppm exhibited central vein dilatation (Plate 4) with enlarged nucleolus and karyorrhexis (Plate 5). Liver section of rats of group IV co-administered with butachlor @262 mg/kg and lead acetate @265 ppm showed congestion of portal area with severe dilatation of central vein (Plate 6) with karyorrhexis and anisocytosis (Plate 7) which 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 changes may be due to the organ responsible

for detoxification of the pesticide which receives massive amounts of metabolites and also due to residual accumulation of it as mentioned by Black *et al.*, 1994 [7] who mentioned that chlorfenapyr acquires insecticidal properties after metabolic activation which functions as un-coupler of oxidative phosphorylation in mitochondria. These results were in agreement with California EPA (2001b) [8] and Federal Register (2003b). Similar finding was studied by Bashir and Noory; 2012 in male albino rat treated with lead.

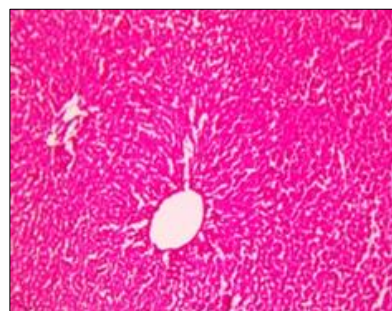


Plate 1: Liver: Group II: Showing hepatocytes degeneration and fatty changes (H & E × 100)

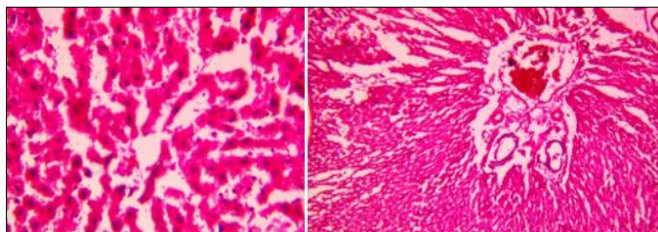


Plate 2 & 3: Liver: Group II: Portal area showing congested portal Vein and mild fibrosis and periportal hepatocytic degeneration (H&E×400, H&E×100 respectively)

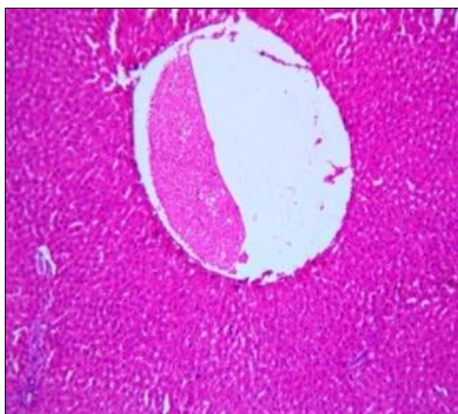


Plate 4: Liver: Group III: dilated central vein (H&E× 100)

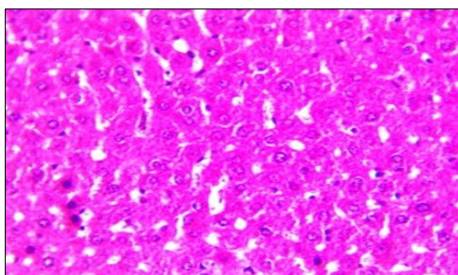


Plate 5: Liver: Group III: Enlargement of nucleolus along with karyorrhexis and moderate hepatocytic degeneration (H & E × 100)



Plate 6: Liver: Group IV: Severe central vein dilation with congested areas H & E × 100

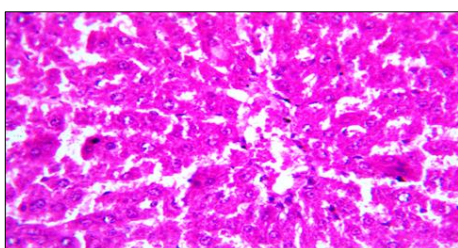


Plate 7: Liver: Group IV: Karyorrhexis, anisocytosis And hepatocytic degeneration H & E × 100

3.2.2. 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. Kidney section of rats of group II administered with butachlor @ 262 mg / kg b. wt. Showed degeneration of tubules and fatty changes mildly (Plate 8). Group III animals administered with lead acetate @ 265 ppm exhibited mild congestion with tubular degeneration (Plate 9). Kidney section of rats of group IV co-administered with butachlor @ 262 mg / kg and lead acetate @ 265 ppm showed moderate degeneration of epithelial cells of PCT, hyper cellularity of glomeruli, and presence of intra tubular hyaline cast (Plate 10) which is in agreement with findings of Ahmadivand *et al.*, 2014 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) [8]. 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) [10].

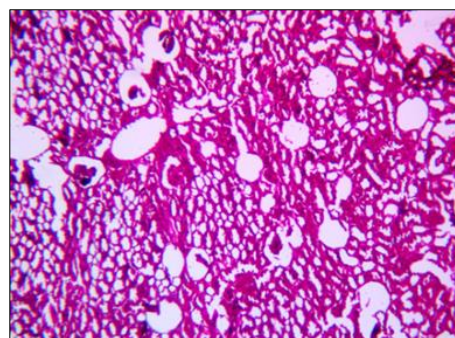


Plate 8: Group II: Showing fatty changes with tubular degeneration (H & E × 100)

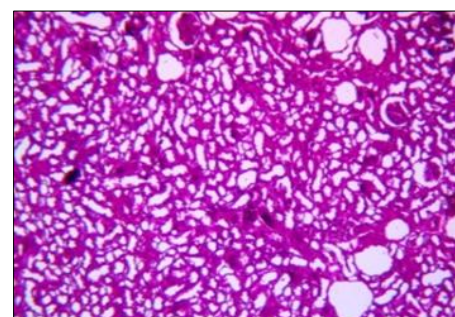


Plate 9: Group III: Showing mild congestion with tubular degeneration (H & E × 100)

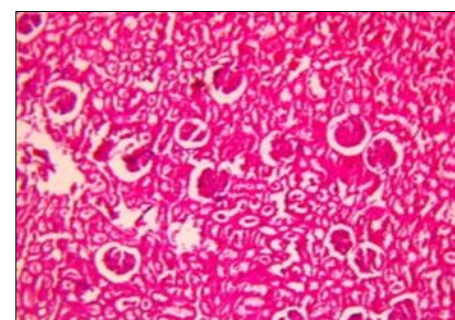


Plate 10: Group IV: Showing moderate hepatocytic degeneration of epithelial cells of PCT, hyper cellularity of glomeruli and presence of intra-tubular hyaline cast (H&E ×100)

4. Conclusion

In the present study, prominent significant changes were assessed due to deleterious effects on liver and kidneys produced by these chemicals alone and in combination. It is of significance to find out toxicological consequences arising as a result of their interaction as such studies help to determine the suitability of use of these chemicals. Studies revealed generation of free radicals during metabolism of these two toxicants in mammalian body. Thus, lead acetate has potentiating effect on the capability of butachlor to induce alterations in activity parameters in rats.

5. Conflict of Interest

The authors declare that there is no conflict of interest.

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