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Effect of repeated oral administration of butachlor and lead alone and in combination on haemato biochemical parameters in wistar rats

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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. Group IV animals showed significant ($p < 0.05$) decrease in levels of Hemoglobin, Packed cell volume, Total erythrocyte count, Lymphocytes, Monocytes levels while significant ($p < 0.05$) increase was observed in Neutrophil, Eosinophils, Basophil, Erythrocyte sedimentation rate. Elevated levels of blood biochemical indices like phosphatases (ACP & ALP), aminotransferases (AST & ALT), Creatinine, BUN was also observed in group IV.

Keywords: Herbicides, butachlor, lead acetate, hematology, biochemical, wistar rats

1. Introduction
Butachlor is a widely used herbicide in rice cultivation and act as a systemic selective pre-emergent herbicide. The mode of action is 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]. Lead, popularly called as "Roman metal" is now a ubiquitous environmental contaminant due to its significant role in modern industry (Shalan *et al.,* 2005) [24]. Lead objects such as sinkers, toys, drapery, weights, printer dye, linoleum, plumbing materials and newspapers are reported to pose risk of lead exposure to humans and animals. In ruminants, the main source of contamination are discarded waste material including batteries, dump oil, oil paint containers, bone-fire ash, lead containing grease, motor vehicle lubricating oil which may lead to accidental lead poisoning. It is one of the most toxic metals known due to its wide ranging effects of multiple body system (Pattee and Pain., 2003) [20], hematological dysfunctions (Patra and Swarup, 2004) [19]. It is a well-known fact that the pesticides form chelating complexes with metals. The assessment of deleterious or toxic effects produced by concurrent exposure to commonly encountered chemicals is of great significance in order to find out toxicological consequences arising as a result of their interactions.

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 with ad libitum feed and water. A daily cycle of 12 h of light and 12 h of darkness was provided to animals. Prior to the start of the experiment, the rats were acclimatized in the laboratory conditions for a period of 2-3 weeks. All the experimental-animals were kept under constant observation during entire period of study. 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 were administered butachlor (Mancheter EC) @ 262 mg / kg B.W / P.O. Group III received administered lead acetate @ 265ppm orally. Animals of Group IV received combination of butachlor @ 262 mg / kg and lead acetate @ 265 ppm.
Blood samples of about 2ml were collected from retro-orbital sinus of rats on day 28th by using capillary-tubes in aliquots containing anticoagulant heparin strength @ 10 IU / ml of blood.

2.1. Haemoglobin: Drabkin solution (5ml) was mixed with whole blood (20 µl) and it was allowed to stand for 5 min. Absorbance was read at 540 nm.

2.2. Packed cell volume (PCV) / Haematocrit value
Micro haematocrit method: A 7 cm long capillary tube with a bore of 1 mm was charged with blood up to ¾ of its length. Capillary tube was placed into slot of haematocrit centrifuge with the sealed end towards outside. Centrifuge was done for 8-10 minutes at 10,000 rpm. After centrifugation, the PCV was read using a PCV reader.

2.3. Erythrocyte Sedimentation Rate (ESR): Win Trobe's method: The Win Trobe's or haematocrit tube was filled with blood up to '0' mark using a Win Trobe pipette. The tube was set in the Win Trobe stand in a vertical position at the constant temperature. The difference between final and initial readings was observed and results were estimated

2.4. Total Erythrocyte Count (TEC): Un-coagulated blood was taken up to 0.5 mark of R.B.C diluting pipette. Diluting fluid was sucked up to 101 mark, 3 or 4 drops of unmixed diluting fluid was discarded from the stem of pipette, and Cells were allowed to settle down for about 1-2 minutes. Chamber was focused under high power microscope. Let, N = the no. Of cells counted in 80 small squares. Then, N/80= the no. Of cells in 1 small squares. The area of 1 small square= 1/400 sq.mm and depth = 1 / 10 mm Volume of 1 small square= 1 / 400 × 1 / 10 cu mm. Then, result is given as the number of cells in 1 cubic mm. N/80 multiplied by 4000. Besides, the blood was originally diluted 1 in 200. Therefore, No. of red cells/cu mm = N × 4000 × 200 / 80.

2.5. Total Leucocyte Count (TLC): A small, measured quantity of blood is diluted with suitable extender like diluting fluid, which will prevent haemolysis of erythrocytes but leave the leucocytes intact. Small drop of this mixture is placed on counting chamber of haemocytometer and number of leucocytes are counted in a known area under a high power microscope (100X) and then calculated in to number of leucocytes per mm^3 of blood, taking the dilution factor into consideration. The Un-coagulated blood is filling up to 0.5 mark of W.B.C diluting pipette. Diluting fluid was sucked up to 11 mark. Pipette was held at 45 ° angles to the surface of counting chamber and tip was placed close to the narrow slit between the counting chamber and the cover slip. Cells were allowed to settle down for about 1-2 minutes. Chamber was focused under high power and W.B.C number were counted in four large (1mm) corner squares of counting chamber under low power microscope with the condenser lowered. Light was adjusted so that leucocytes appear as round, slightly indented bodies with a definite outline. Leucocytes had definite cell outlines and defined nuclei. Platelets appeared as very small refractive bodies about 1 / 5th to 1 / 7th size of leucocytes without nuclei. Let the total number of W.B.C present in four squares of 1 sq. mm area of 1/10 mm depth i. e, 4 × 0.1 cu mm area = A1 + A2+ A3 +A4 = X. The no. of W.B.C present in 1 sq. mm area of 0.1 mm depth i.e., in 0.1 cu.mm. Volume = X / 4.The no. of W.B.C present in 1 cu.mm volume = X / 4 × 10. The original blood sample is diluted 20 times that is 0.5 parts in 10 (11-1) parts. Therefore, 1 cu.mm of undiluted blood contains X / 4 ×10×20 i.e. X × 50.

2.6. Differential Count of Leucocytes (DLC): It may be defined as number of various types of leucocytes per hundred WBC counted.

2.7 Preparation of Blood Smear: A small drop of fresh or anticoagulant mixed blood was placed on clean, grease free, dry glass slide about 2cm away from one end. Another slide (spreader slide) was placed in front of the blood drop such that an angle of 30-45° was made between the two slides. With a single swift forward movement of spreader slide, the blood was spread along the surface of slide. Smear was dried immediately by waving the slide rapidly or exposing it to air. The rapid drying of smear prevents crenation of cells. The dried smears were stained on the staining rack with Leishman's stain or Wright stain**.** Stain was allowed to act for 1-2 minutes at room temp. Equal quantity of distill water or phosphate buffer was added and thoroughly mixed with the stain either by rocking movement or blowing air through a glass tube. Incubation was done for 5-10 minutes at room temperature. Stain solution was washed off gently with distilled water and then dried. Slide was focused under high power microscope to see the distribution of cells and nature of staining.

2.8. Biochemical analysis: Immediately after collection of blood, samples were centrifuged at 3000 rpm for 15 minutes to harvest the plasma and then kept in clean sterile glass test tubes and stored at -20 ºC for further biochemical analysis. Standard kits procured from Recon diagnostics Pvt. Ltd / Trans Asia Bio-Medicals were used for determination of all biochemical parameters in this study. Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by using standard kits from Trans Asia Bio-Medicals. The principle reaction of the calorimetric determination of AST and ALT activity is based on the reaction of aspartate or alanine with α-ketoglutrate to form oxaloacetate or pyruvate, respectively. The oxaloacetate or pyruvate formed was measured by monitoring the concentration of oxaloacetate or pyruvate hydrazone formed with 2, 4-dinitrophenyl hydrazine. Plasma alkaline and acid phosphatase activity was measured at 405 nm by the formation of para-nitrophenol from para-nitrophenyl phosphate as a substrate. Urea in blood reacts with water in presence of urease and forms ammonia which again reacts with α-ketoglutrate and NADH in presence of glutratedehydogenase to form glutamate and NAD which was measured spectrophotometrically at 500-520nm.Creatinine reacts with alkaline picrate to produce an orange yellow colour, the absorbance is directly related to creatinine concentration and was measured spectrophotometrically at 500-520nm.

3. Results
3.1. Haematological alterations
3.1.1. Haemoglobin: A statistically significant ($p < 0.05$)

decrease in haemoglobin level was observed in group II (12.69±0.80) but non-significant change was observed in group III (13.20±0.97) as compared to the control group I (14.70±0.96). In contrast, combination groups: group IV (9.96±0.65) showed significant ($p < 0.05$) decrease in HB level as compared to control group I (14.70±0.96). [Table 01; Fig. 01].

3.1.2. Packed cell volume (PCV): A statistically significant ($p < 0.05$) decline in packed cell volume level was observed in combination group IV (29.76±2.48) but non-significant decrease was observed in group II (38.07±2.42); group III (39.59±2.93) as compared to control group I (44.13 ± 4.04) [Table 01; Fig. 01].

3.1.3. Total Leukocyte count (TLC): A statistically significant ($p < 0.05$) elevation in total leukocyte count was observed in group II, group III (15.0±0.73); (12.5±0.84) as compared to control group I (9.33±0.66). However combination group IV (16.1±0.94) showed significant rise as compared to control group I. [Table 01; Fig. 01].

3.1.4. Total Erythrocyte Count A statistically significant ($p<0.05$) decrement in total erythrocyte count was observed in group II (4.7±0.31); group III (5.3±0.42) as compared to control group I (6.8±0.30). Combination groups IV (3.6±0.33) showed significant ($p < 0.05$) decrease as compared to control group I. [Table 01; Fig. 01].

3.1.5. Erythrocyte Sedimentation Rate: A statistically significant ($p < 0.05$) increase in erythrocyte sedimentation rate was observed in group II (3.8±0.4) as compared to control group I (2.3±0.21). Group III (3.5±0.22) showed non-significant increase as compared to control group I (2.3±0.21). Combination group: group IV (5.7±0.34) showed significant increment as compared to control group I [Table 01; Fig. 01].

Table 1: Effect of oral administration of butachlor and lead acetate alone and in combination on blood profile (Hb, PCV,TLC,TEC,ESR) in wistar rats (n = 6).

Group	Treatment	HB (g/dl)	PCV (%)	TLC(thousands/µl)	TEC(millions/µl)	ESR(mm/hr)
I	Control	14.70^{c}±0.96	44.13^{d}±4.04	9.33^{a}±0.66	6.8^{e}±0.30	2.3^{a}±0.21
II	Butachlor @ 262 mg / kg	12.69^{bc}±0.80	38.07^{bcd}±2.42	15.0^{cd}±0.73	4.7^{c}± 0.29	3.8^{b}±0.40
III	Lead @265 ppm	13.20^{c}±0.97	39.59^{cd}±2.93	12.5^{bc}±0.84	5.3^{cd}±0.42	3.5^{ab}±0.22
IV	Butachlor @ 262 mg / kg + Lead @265 ppm	9.96^{ab}±0.65	29.76^{ab}±2.48	16.1^{d}±0.94	3.6^{b}±0.33	5.7^{c}±0.34

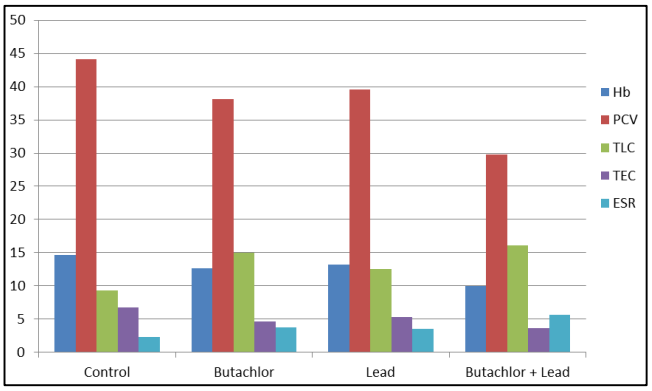

Fig 1: The effect of sub-acute oral exposure of butachlor and lead acetate alone and in combination on mean values of blood profile (Hb, PCV, TLC, TEC, ESR) in wistar rats (n=6)

3.2. Differential leukocytic count (DLC)

3.2.1. Lymphocyte: A statistically significant ($p < 0.05$) decline in lymphocytes was observed in combination group IV (55.1±4.66) as compared to control group I (68.3±4.07) but non-significant decrease was observed in group II (59.6±3.09); group III (62.6±3.14); as compared to control group I [Table 02; Fig. 02].

3.2.2. Neutrophil: A statistically significant ($p < 0.05$) increase in neutrophils was observed in group IV (24.66±4.2) as compared to control group I (24.66±4.2) but groups which were exposed to toxicant alone: group II (32.16±2.7); III (29.00±2.9) showed non-significant increase as compared to control group I (24.66±4.2) [Table 02; Fig. 02].

3.2.3. Monocyte: A statistically significant decrease ($p < 0.05$) in monocytes was observed in group II (2.1±0.17) but group III (2.3±0.21) showed non-significant decrease as compared to control group I (2.8±0.16). Combination group IV (1.6±0.21) showed significant ($p < 0.05$) decrease as compared to control group I (2.8±0.16) [Table 02; Fig.02].

3.2.4. Eosinophil: A statistically significant ($p < 0.05$) rise in eosinophils was observed in all groups as compared to control group I (3.0±0.25). [Table 02; Fig. 02].

3.2.5. Basophil: A statistically significant ($p < 0.05$) fall in basophils was observed in group IV (2.0±0.25) but a non-significant decrease was observed in group II (1.5±0.22); III (1.33±0.21); V (1.66±0.21) as compared to control group I (1.16±0.61). [Table 02; Fig. 02].

Table 2: Effect of oral administration of butachlor and lead acetate alone and in combination on differential leukocytic count in wistar rats (n = 6)

Group	Treatment	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
I	Control	68.3^{d}±4.07	24.66^{a}±4.2	2.8^{d}±0.16	3.0^{a}±0.25	1.16^{a}±0.16
II	Butachlor @ 262 mg / kg	59.6^{abcd}±3.09	32.16^{abcd}± 3.1	2.1^{bc}±0.10	4.5^{bc}±0.22	1.5^{ab}±0.22
III	Lead @ 265 ppm	62.6^{bcd}±3.14	29.00^{abc}±2.9	2.3^{cd}±0.29	4.6^{bc}±0.35	1.3^{a}±0.11
IV	Butachlor @ 262 mg / kg + Lead @ 265 ppm	55.1^{abc}±4.66	37.16^{bcd}±4.3	1.6^{ab}±0.20	5.0^{c}±0.41	2.0^{bc}±0.25

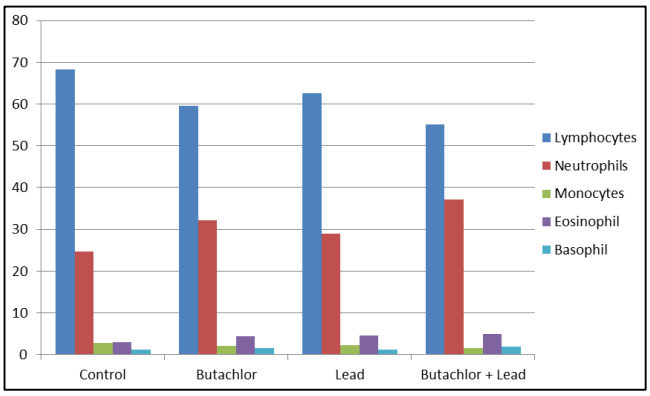

Fig 2: The effect of subacute oral exposure of butachlor and lead acetate alone and in combination on mean values of differential leukocytic count (Lymphocyte, Neutrophil, Monocyte, Eosinophil, Basophil) in wistar rats (n = 6).

3.3. Biochemical alterations

3.3.1. Plasma amino transferase: A statistically significant ($p < 0.05$) increase in AST levels were observed in group II (147.63±7.40); group III (143.15±5.45). The groups which were exposed to combination of toxicants i.e. group IV (156.5±3.4) showed significant increase ($p < 0.05$) in AST activity as compared to control. A Significant ($p < 0.05$) increase in ALT activity was observed in group II (75.65±4.7); group III (73.55±2.3) showed non-significant increase as compared to control (52.32±2.8). Combination groups: group IV (87.138±5.775) showed significant increase ($p < 0.05$) in ALT activity. [Table 03, Fig. 03].

3.3.2. Plasma phosphatase: A statistically significant ($p < 0.05$) increase in acid phosphatase (ACP) levels were observed in group II (61.60±3.4), group III (57.38±2.4) as compared to control group I (41.05±3.9). The groups which were exposed to a combination of toxicant i.e. group IV (77.600±3.907) showed significant increase ($p<0.05$) in ACP activity. A significant ($p<0.05$) increase in alkaline phosphatase (ALP) levels was observed in all groups II (377.037±25.127); III (358.978±20.253), IV (460.700±23.939) as compared to control group I (270.607±25.353). [Table 03, Fig. 03].

3.3.3. Blood Urea Nitrogen (BUN) and Creatinine (CRE): A statistically significant ($p < 0.05$) increase in blood urea nitrogen (BUN) levels were observed in group II (56.02±3.42); group III (47.94±3.26) as compared to control (23.08 ± 2.17). The groups which were exposed to a combination of toxicant i.e. group IV (67.873±3.308) showed significant rise ($p<0.05$) in BUN activity. A significant ($p < 0.05$) rise in creatinine (CRE) levels were observed in group II (0.992±0.041); III (0.965±0.062); IV (1.013±0.109) as compared to control (0.593^{a}±0.036). [Table 03, Fig. 03]

Table 3: Effect of oral exposure of butachlor and lead acetate alone and in combination on mean values of hepatic biomarkers (ACP,ALT,ACP,ALT) and renal biomarkers (BUN, CRE) in wistar rats (n = 6).

Group	Treatment	AST (units / l)	ALT (units / l)	ACP (units / l)	ALP (units / l)	BUN (mg / dl)	CRE (mg / dl)
I	Control	114.77^{a}±6.76	52.32^{a}±2.8	41.05^{a}±3.9	270.60^{a}±25.35	23.08a±2.17	0.593^{a}±0.036
II	Butachlor @ 262 mg / kg	147.63^{cd}±7.40	75.65^{bc}±4.7	61.60^{cd}±3.4	377.03^{cd}±25.12	56.02^{bc}±3.42	0.992^{b}±0.041
III	Lead @ 265 ppm	143.15^{bc}±5.45	73.55^{bc}±2.3	57.38^{bc}±2.4	358.97^{bc}±20.25	47.94^{b}±3.26	0.965^{b}±0.062
IV	Butachlor @ 262 mg / kg + Lead @ 265ppm	156.59^{cd}±13.4	87.13^{cd}±5.7	77.60^{e}±3.9	460.70^{d}±33.93	67.87^{d}±4.30	1.013^{b}±0.109

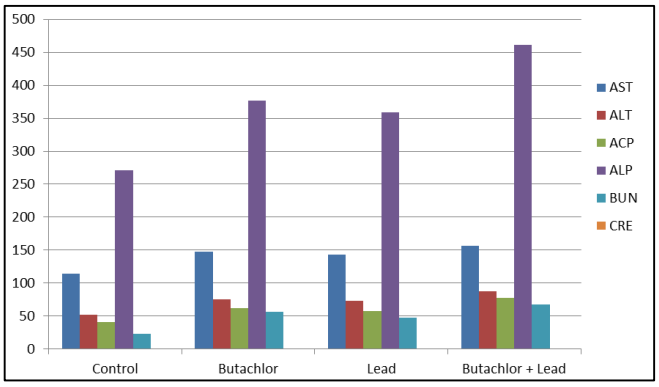

Fig 3: The effect of sub-acute oral exposure of butachlor and lead acetate alone and in combination on mean values of hepatic biomarker and renal biomarker activity in wistar rats (n=6).

4. Discussion

In the quest for safe and efficacious agricultural chemical protectants, novel pesticides are introduced at frequent intervals. However, no pesticide has been developed till date which is not contaminating the environment and is completely safe and free from the inherent degree of toxicity to non-target species. It is also a well-known fact that the pesticides form chelating complexes with metals. The usage of effluents for agriculture purposes leading to contamination of food with heavy metals has been reported. As a consequence, both heavy metals and pesticides can enter different tropic levels of food chain due to primary uptake by plants. The assessment of deleterious or toxic effects produced by concurrent exposure to commonly encountered chemicals is of great significance in order to find out toxicological consequences arising as a result of their interactions. In our present study, a herbicide and metal was administered alone and combination with the aim to find the possible sub-acute deleterious effect posed to environment by the human due to the indiscriminate use of the chemicals.

4.1. Haematological alterations: Our present finding is in agreement to Hussain's, 2014 [10] finding which showed significant reduction in RBC counts, haemoglobin concentration and haematocrit percent in Japanese quail. The decline in RBC cell counts could be due to the toxic effects of herbicide on circulating erythrocytes and the blood forming tissues. The decrease in haemoglobin levels could also be due to toxic effects of butachlor on maturating erythrocytes and inadequate iron supply (Hussian, 2011) [30] while decrease in haematocrit values could be due to increase destruction of erythrocyte or reduction in size (Rahman, 2006) [22]. ESR is negatively correlated with total erythrocyte count. A lower total erythrocyte count reciprocates with a higher ESR value (Agarwal and Chaurosia, 1989) [2]. An increase in ESR (mm / hr) may be due to an increase in the concentration of fibrinogen which results in fibrinogenemia as result of chlorpyrifos exposure (Singh and Bhatia, 1991) [25]. The term

WBC pertains to all types of leukocytes, including granulocytes (neutrophils, eosinophil, basophils), lymphocytes and monocytes. Repeated exposure butachlor to rat resulted in significant increase in leucocyte count, and these observations are in agreement to the study of butachlor by Joshi *et al.*, 2003 [11]. Ahmadivand, 2014 [3] studied reduction in lymphocyte values could be caused by the effects of butachlor as an anti-androgenic endocrine disruptor, because androgen plays an important role in haematological homeostasis by mediating lymphocyte proliferation (Milla *et al*, 2011) [16]. Conversely butachlor exposure resulted in an enhancing effect on neutrophil values in fish. This may be due to ability of butachlor to induce an immune response. It has been shown that neutrophil and macrophages activator gene IL-1b is significantly induced by butachlor in embryonic zebra fish (Tu *et al*, 2013) [28]. In current study a significant reduction in RBC, PCV, Hb has been observed following exposure of lead acetate in rats resulting in microcytic hypochromic anaemia. These haematological alterations may be due to the effect of lead on activity of aminolevulinic acid dehydratase (ALAD), a key enzyme of heme synthesis. Ekanem *et al*, 2015 [32] studied effect of lead acetate on spleen and blood parameters in albino rats and showed decline in Hb. contents below the normal values due to decrease in the values of total erythrocyte count (Marchetti, 2003) [15]. This may be due to the adverse effects of the lead acetate on the haematopoietic system and on absorption of essential vitamins and minerals from the intestines as well as the destruction of the red blood cells in the body of animals. Alwaleedi, 2015 [4] studied effect of lead acetate in mice and showed significant reduction in the total erythrocytic count (TEC) following exposure of lead acetate, also observed marked decrease in the levels of haemoglobin and packed cell volume. As such individually Butachlor and Lead affected hematopoiesis adversely, and reduced the levels of haemoglobin, packed cell volume and total erythrocyte count, and in turn increased the levels of erythrocyte sedimentation rate to their respective groups. The group exposed to Butachlor plus Lead exhibited added toxicity which proved to be significantly more toxic in comparison to Butachlor and Lead (alone) exposed groups.

4.2. Biochemical alterations

4.2.1. Plasma Phosphatases: The increase in acid phosphatase enzyme activity could be explained on the basis of enhancement of cell membrane permeability with disturbance in the Trans phosphorylation process as a result of cellular degeneration (Linder *et al*., 1998) [33]. Decrease in AKP activity may be taken as an index of hepatic parenchymal damage and hepatocytic necrosis (Onikienko, 1963) [18]. Inhibition of AKP reflects alterations in protein synthesis and uncoupling of oxidative phosphorylation (Verma *et al*., 1984) [34]. Similar results were recorded in previous studies following repeated Chlorpyrifos exposure (Ambali *et al*., 2007; Ambali, 2009 and Goel *et al*., 2005) [6, 5, 8]. Alwaleedi, 2015 [4] studied the effect of lead acetate on mice and reported increase in serum AKP activity and explained it might due to damage caused to liver, kidney, and bone leading to pouring of AKP (Kaplan and Reghetti, 1970). These results are in accordance with other findings (Shalan *et al*, 2005).

4.2.2. Plasma aminotransferases: Alanine transaminase (ALT) concentration is highest in liver. So, it is more sensitive marker to hepatocellular damage than AST (Lin *et al*, 1997); (Muhammad, 2012). The increase in the activity of plasma ALT is mainly due to leakage of this enzyme from liver cytosol into the blood stream, which reflects liver damage and disruption of normal liver function (Shakoori *et al*., 1994) [23]. Hussain, 2014 [10] found similar result with Butachlor in Japanese quail, the enzyme AST and ALT significantly increased. Eman *et* al. (2008) [31] observed rise in phosphatase activity in plasma of rats exposed to chlorfenapyr. Suradkar, 2009 [26] found similar result in wistar rats with lead acetate, this might be due to increased cell membrane permeability or cell membrane damage of hepatocytes caused by lead acetate. These findings are in accordance with Shalan *et al*., 2005 [24]. Lead has hepatotoxic effect leading to hepatocellular damage which causes increase in serum levels of AST and ALT (Abdou *et al.*, 2007) [1]. It has been observed that lead has toxicological effects on rat liver leading to liberation of AST and ALT in to blood stream (Shalan *et al.*, 2005) [24].

4.2.3. Blood urea nitrogen and Creatinine: Increase in the BUN and CRE levels suggest impairment in glomerular function and reduced ability of kidney to eliminate the toxic metabolic substances (George et al., 2014) [7]. This increase in creatinine concentration has been correlated to loss of 50% of kidney function and considered as functional evidence of lead induced nephrotoxicity (Qu *et al*., 2002) [21]. Joshi *et al*., 2012 [12] also reported increase in blood urea following administration of butachlor which may be due to high concentration of non-protein nitrogenous substances due to inability of the body system to eliminate the metabolic end product of protein (Tomito, 2006) [27]. Similar finding was reported by Alwaleedi, 2015 [4] after administration of lead acetate in male and female albino mice, and this surge in creatinine has been attributed to kidney dysfunction and considered as functional evidence of lead induced nephrotoxicity (Zook, 1972) [29].

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