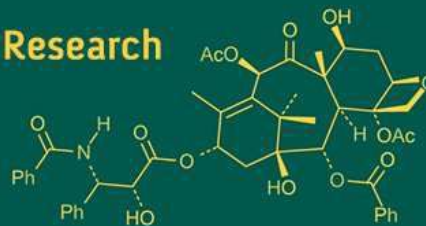


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Effect of subacute exposure of Pretilachlor in black Bengal goats

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Abstract

This study investigated the subacute toxic effects of pretilachlor on hematological, biochemical, and histopathological parameters in Black Bengal goats. Erythrocytic and leucocytic indices showed no significant changes, except for a non-significant reduction in hemoglobin (Hb) levels and lymphocyte count. Oxidative stress, indicated by elevated malondialdehyde (MDA) levels, was observed in the blood and tissues of exposed goats. Antioxidant defense enzymes such as superoxide dismutase (SOD) and catalase increased their activity to counteract free radicals. A significant reduction in reduced glutathione (rGSH) levels in the kidney suggested stress in renal tissue due to pretilachlor exposure. Blood glucose levels remained unchanged, while tissue protein levels decreased, and tissue enzyme markers such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) increased in the liver and heart, indicating stress. Histopathological examination revealed Zenker's degeneration and fatty infiltration in the heart and hydropic degeneration in the kidney, supporting biochemical findings. Residue analysis showed higher pretilachlor levels in the blood over time, with the gastrointestinal tract having the highest residue levels. Significant residues were also found in the heart, lungs, adrenal glands, muscles, fat, liver, and kidneys, indicating pretilachlor accumulation in these organs.

Keywords: Pretilachlor, black Bengal goats, hematology, biochemical, tissue, histopathology, residue analysis

Introduction

Advances in agriculture continue to rely on pesticides for plant protection, significantly boosting productivity. A large portion of India's population is engaged in agriculture, making them susceptible to the harmful effects of pesticide exposure [1]. Herbicides, a major class of pesticides, are used to eliminate weeds that compromise crop growth and yield, particularly in row-crop farming to enhance productivity by reducing competing vegetation.

Pretilachlor (PTC), chemically 2-chloro-2', 6'-diethyl-N-(2-propoxyethyl) acetanilide, is a widely used broad-spectrum systemic herbicide in India and Southeast Asia. It is effective against annual weeds, sedges, and broadleaf weeds in rice fields. PTC can be detected in surface water, soil, and sediments. It inhibits long-chain fatty acid synthesis by targeting elongase enzyme, thus hindering cell division in plants [2]. PTC also affects the synthesis of proteins, nucleic acids, lipids, gibberellic acid, and geranyl pyrophosphate cyclization enzymes [3, 4]. Research indicates that PTC reduces weed density and increases rice yields [5]. However, improper use can alter soil biochemical and microbial properties, affecting non-target organisms such as fish [6]. Pretilachlor exposure in fish, such as *Ctenopharyngodon idella*, causes gill damage, hemorrhage, hyperemia, hyperplasia, cell necrosis, and increases blood glucose and cortisol levels [7]. In rats, PTC induces phase I and II xenobiotic-metabolizing enzyme activities selectively [8].

To assess the impact of PTC on non-target terrestrial animals, a study was conducted on female Black Bengal goats. This short-term study evaluated the cumulative toxicity of repeated doses of PTC, using both minimum toxic and maximum non-toxic doses to understand dose-dependent variations in various parameters.

Materials and Methods

Technical grade pretilachlor (> 92% purity) was procured from Monsanto Pesticide chemical Company, West Bengal, India.

All other chemicals were obtained from E. Merck (India), Rankem (India), and Sigma Chemicals Co. (USA).

Fixation of dose in case of Pretilachlor

Eight female goats were divided into four groups. The first group received an oral administration of the vehicle, 0.5% DMSO (3 ml). The remaining three groups were administered pretilachlor orally at doses of 152.5, 305, and 610 mg/kg body weight (BW) on the basis of literature review. The goats given 610 mg/kg BW died after 7 days, exhibiting signs of anorexia, dullness, depression, and asymptomatic diarrhea. Those administered 305 mg/kg BW displayed toxic symptoms, including anorexia, dullness, and depression, throughout the experimental period. Therefore, 305 mg/kg BW was identified as the minimum toxic dose, while the lower dose of 152.5 mg/kg BW, which did not induce any toxic symptoms, was considered the maximum non-toxic dose of pretilachlor.

Animals

Clinically healthy female Black Bengal goats (N=20), weighing between 8-12 kg and aged approximately 1.5-2 years, were used for this experiment. Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) approvals were obtained prior to the study. The goats were maintained under standard laboratory conditions and dewormed with a single oral dose of rafoxanide (7.5 mg/kg BW) 30 days before the study commenced. The goats were divided into two main groups: Group A (4 goats) and Group E (16 goats). Group E was further divided into two subgroups (I and II) with 8 goats each. Subgroup I was administered the minimum toxic dose, and Subgroup II received the maximum non-toxic dose of pretilachlor daily for 28 days. Four animals from each subgroup were sacrificed on the 29th day of the experiment, and the remaining four animals were sacrificed at the end of a 15-day withdrawal period.

Sample Collection

Blood samples were collected from the jugular vein of each goat in all groups on the 0th, 14th, and 28th days of the experiment for hematological studies, residue analysis, biochemical studies, and glucose estimation. Two milliliters of blood were clotted for serum separation and stored at -20°C for assays of AST, ALT, serum protein and other biochemical parameters. For oral disposition kinetics, blood samples were collected from the right jugular vein at 0, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 36, 48 and 72 hours post-administration of the pesticide.

Tissue samples were collected from four animals in each group, sacrificed on the 29th day of the experiment, and the remaining four animals on the 43rd day (15 days post-dosing withdrawal period). Organs collected included the liver, kidney, heart, lung, skeletal muscle, spleen, fat, adrenal gland, brain, uterus, bile, skin, large and small intestines, rumen, reticulum, omasum, abomasum, bone, mammary gland, urinary bladder, and subscapular lymph node for residue estimation of pretilachlor. Portions of the liver, heart, and kidney were used to estimate tissue biochemical parameters, and the caudate lobe of the liver was used for cytochrome P450 estimation. Tissue homogenates (10% and 5%) were prepared for analyzing catalase, total protein, AST, ALT, and other parameters.

Standard procedures were followed for all parameter estimations.

Methods from Schalm's Veterinary Hematology were used to estimate hemoglobin, total erythrocyte count (TEC), packed cell volume (PCV), total leukocyte count (TLC), and differential leukocyte count (DLC) [9]. Serum protein was measured using the Biuret method [10], and blood glucose was determined with a Glucose Assay kit using the GOD-POD method [11]. Serum alanine and aspartate aminotransferase activities were measured with a COGENT kit based on the 2,4-DNPH method [12] and lipid peroxidation was estimated following standard protocols [13]. For blood biochemical parameters, a 5% hemolysate was prepared for hemoglobin [14] superoxide dismutase [15] catalase [16] and reduced glutathione analyses [17]. Liver tissue homogenates (10%) were used for lipid peroxidation estimation [18] and 5% homogenates for catalase activity measurement. Cytochrome P450 estimation followed established methods with modifications [19]. Portions of the liver, kidney, heart, spleen, and lymph nodes from both control and experimental goats were fixed in 10% formal-saline for histopathological studies [20].

Concentration of pretilachlor

Quantitative estimation of pretilachlor was conducted using a Varian 3800 gas chromatography system (Varian Inc., USA) with a 63Ni electron capture detector (ECD). Chromatographic separation was achieved on a 30 m x 0.25 mm DB-5MS capillary column with a 0.25 µm film thickness (Agilent J and W Scientific). High-purity nitrogen served as both carrier and make-up gas, with flow rates of 1 ml/min and 25 ml/min, respectively. The on-column injector was set at 275 °C, the oven at 210 °C, and the detector at 300 °C. Data acquisition and chromatogram management were performed using a Galaxie Workstation. Manual injections were made with a 10 µl SGE micro syringe.

For pretilachlor estimation, a 1 µl sample was injected into the GC injection port, with the first and last samples serving as external standards. Pretilachlor concentrations in blood and tissues were determined using a calibration curve and calculated in µg/ml or µg/g using the formula:

$$\text{Residue (ppm)} = \frac{C \times V}{M}$$

where:

C is the concentration from the calibration curve (µg/ml)

V is the final volume of the processed sample (ml)

M is the volume or weight of the sample used for extraction (g or ml)

Results and Discussion

The data as evident from Table 1 indicate no significant changes in hemoglobin levels, total erythrocyte count (TEC), or packed cell volume (PCV) in goats administered pretilachlor at 152.5 and 305 mg/kg BW for 28 days. A non-significant reduction in hemoglobin values was noted on day 28 compared to day 0. This decrease may be attributed to inhibited erythropoiesis, impaired haemo-synthesis, osmoregulatory dysfunction, or increased erythrocyte destruction due to herbicide exposure. Similar results were reported showing significant reductions in RBCs, Hb, HCT, and PL in *C. gariepinus* fish exposed to acetochlor [21]. Significant reductions in erythrocyte indices in fish exposed to butachlor were also documented [22]. Blood parameters are

reliable indicators of the physiological impact of toxicants, and changes in these biomarkers reflect the organism's physiological status. The decreased hemoglobin content may result from impaired metabolic and hemopoietic activities in goats exposed to pretilachlor. Similarly, pretilachlor did not significantly affect leucocytic indices, as shown in Table 2, except for a non-significant reduction in lymphocyte percentage on day 28 compared to day 0. Neutrophil counts remained unaffected. Similar studies reported significant decreases in erythrocyte count, packed cell volume, hemoglobin, platelets, lymphocytes, and mean corpuscular volume in *Clarias gariepinus* fish exposed to atrazine and metolachlor mixtures [23]. A decline in WBC and lymphocyte indices was also observed in common carp (*Cyprinus carpio* L.) exposed to terbutylazine and S-metolachlor²⁴. The reduction in lymphocyte count may be linked to stress from pesticide exposure.

Table 1: Effect of Pretilachlor on erythrocytic indices in goats following 28 days of daily oral administration at two dose levels (152.5 and 305 mg/kg BW) (Mean $\pm$ SE of 4 Replicates)

Parameters	Days Groups	0	14	28
Hemoglobin (g/dl)	A	8.82 $\pm$ 0.45	8.92 $\pm$ 0.46	8.90 $\pm$ 0.54
	EI	8.92 $\pm$ 0.40	8.82 $\pm$ 0.36	8.75 $\pm$ 0.32
	EII	8.72 $\pm$ 0.32	8.57 $\pm$ 0.46	8.32 $\pm$ 0.27
TEC ($\times 10^{12}$ /L)	A	12.03 $\pm$ 1.94	12.25 $\pm$ 1.75	12.14 $\pm$ 1.73
	EI	12.16 $\pm$ 1.51	12.02 $\pm$ 1.51	11.96 $\pm$ 1.70
	EII	12.26 $\pm$ 1.60	11.98 $\pm$ 1.43	11.04 $\pm$ 1.70
PCV (%)	A	25.50 $\pm$ 2.39	26.00 $\pm$ 2.27	26.25 $\pm$ 2.21
	EI	24.00 $\pm$ 2.14	22.50 $\pm$ 1.89	22.25 $\pm$ 2.09
	EII	25.50 $\pm$ 1.93	24.00 $\pm$ 2.27	23.00 $\pm$ 2.04

Group A; control group administered with DMSO

Group BI and BII administered with 152.5 and 305 mg/kg BW pretilachlor daily orally for 28 days

Table 2: Effect of Pretilachlor on leucocytic indices in goats following 28 days of daily oral administration at two dose levels (152.5 and 305 mg/kg BW) (Mean $\pm$ SE of 4 Replicates)

Parameters	Days Groups	0	14	28
TLC ($\times 10^9$ /L)	A	14.47 $\pm$ 0.94	15.64 $\pm$ 1.02	14.66 $\pm$ 1.60
	EI	14.60 $\pm$ 0.98	13.98 $\pm$ 1.07	13.99 $\pm$ 0.91
	EII	15.39 $\pm$ 0.89	15.01 $\pm$ 0.98	14.21 $\pm$ 1.17
Lymphocytes (%)	A	66.90 $\pm$ 1.81	68.07 $\pm$ 1.25	68.75 $\pm$ 1.36
	EI	65.18 $\pm$ 1.56	64.87 $\pm$ 1.67	64.77 $\pm$ 1.73
	EII	67.52 $\pm$ 1.29	66.77 $\pm$ 1.58	65.54 $\pm$ 1.65
Eosinophils (%)	A	10.55 $\pm$ 0.82	10.20 $\pm$ 1.13	10.37 $\pm$ 0.78
	EI	10.45 $\pm$ 0.66	10.10 $\pm$ 0.86	10.03 $\pm$ 0.81
	EII	10.11 $\pm$ 0.97	10.05 $\pm$ 0.81	9.88 $\pm$ 0.65
Neutrophils (%)	A	48.25 $\pm$ 1.54	49.43 $\pm$ 1.90	49.79 $\pm$ 1.74
	EI	47.84 $\pm$ 1.79	47.19 $\pm$ 1.82	46.58 $\pm$ 1.57
	EII	49.53 $\pm$ 1.62	49.94 $\pm$ 1.35	48.78 $\pm$ 1.48

Group A; control group administered with DMSO

Group EI and EII; administered with 152.5 and 305 mg/kg BW pretilachlor daily orally for 28 days

The serum protein levels showed no significant differences throughout the experimental period. However, a significant increase in serum ALT and AST levels was observed in group EII on day 28 compared to day 0 as depicted in figure 1a and 1b. Changes in plasma enzyme activity, such as ALT and AST, serve as biomarkers for tissue injury, environmental stress, or disease, indicating liver damage when elevated. Pretilachlor exposure in group EII increased these enzyme levels after 28 days, similar to findings in Common Carp, where pretilachlor exposure elevated AST, ALT, and ALP levels [25]. The increase in ALT and AST activities in goats' serum may be due to pretilachlor-induced liver toxicity, leading to hepatocyte necrosis and enzyme leakage into the bloodstream [26]. Elevated serum AST and ALT in *C. gariepinus* fish exposed to acetochlor was also seen [21]. ALT levels in the liver and heart of group EII were significantly higher than in the control group, with heart ALT levels also elevated compared to group EI as depicted in figure 2a. Figure 2b depicts, increased AST levels in the

heart and kidney tissue compared to the control group, with kidney AST levels also higher than in group EI. This increase in liver enzyme levels can be linked to the metabolism of keto acids in the tricarboxylic acid cycle, generating energy through tissue transamination and oxidative deamination [27]. The mean values of tissue protein depicted in figure 2(c) showed a significant decline in liver tissue, likely due to stress from xenobiotic exposure. Histopathological findings showed Zenker's degeneration in the heart and fatty infiltration and hydropic degeneration in the kidney, supporting the biochemical data. Sub-chronic exposure to glyphosate herbicides in Wistar rats led to increased serum ALT and AST, suggesting irreversible hepatocyte damage [28]. Similarly elevated AST and ALT levels in amphibian tadpoles exposed to Dicamba was also reported [29]. In contrast to our findings, decreased AST and ALT levels in *Labeo rohita* fish exposed to PTC was also reported [30].

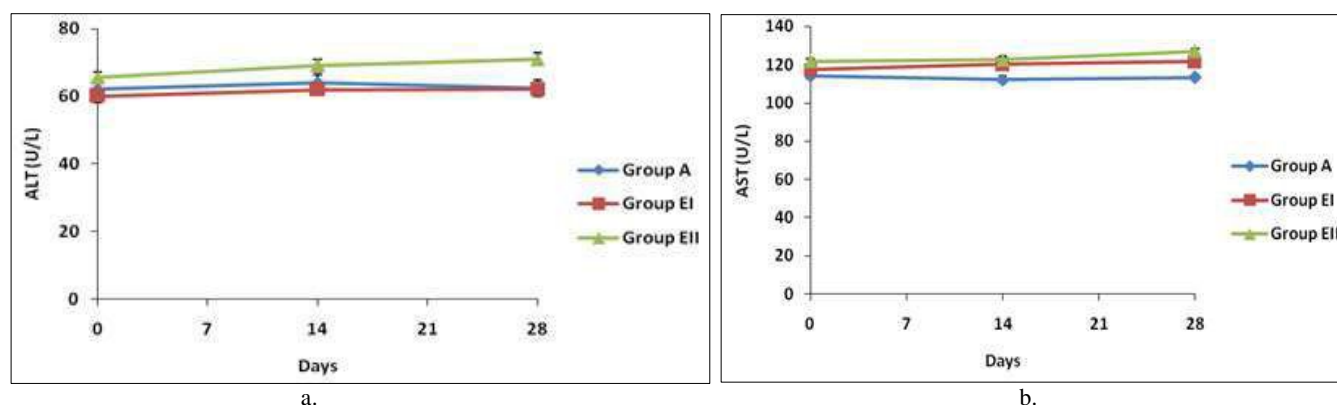


Fig 1 a, b: Effect of pretilachlor on serum ALT and AST (U/L) values in goats following daily oral administration at two dose levels (152.5 and 305 mg/kg BW) for 28 days. (Mean $\pm$ SE of 4 replicates)

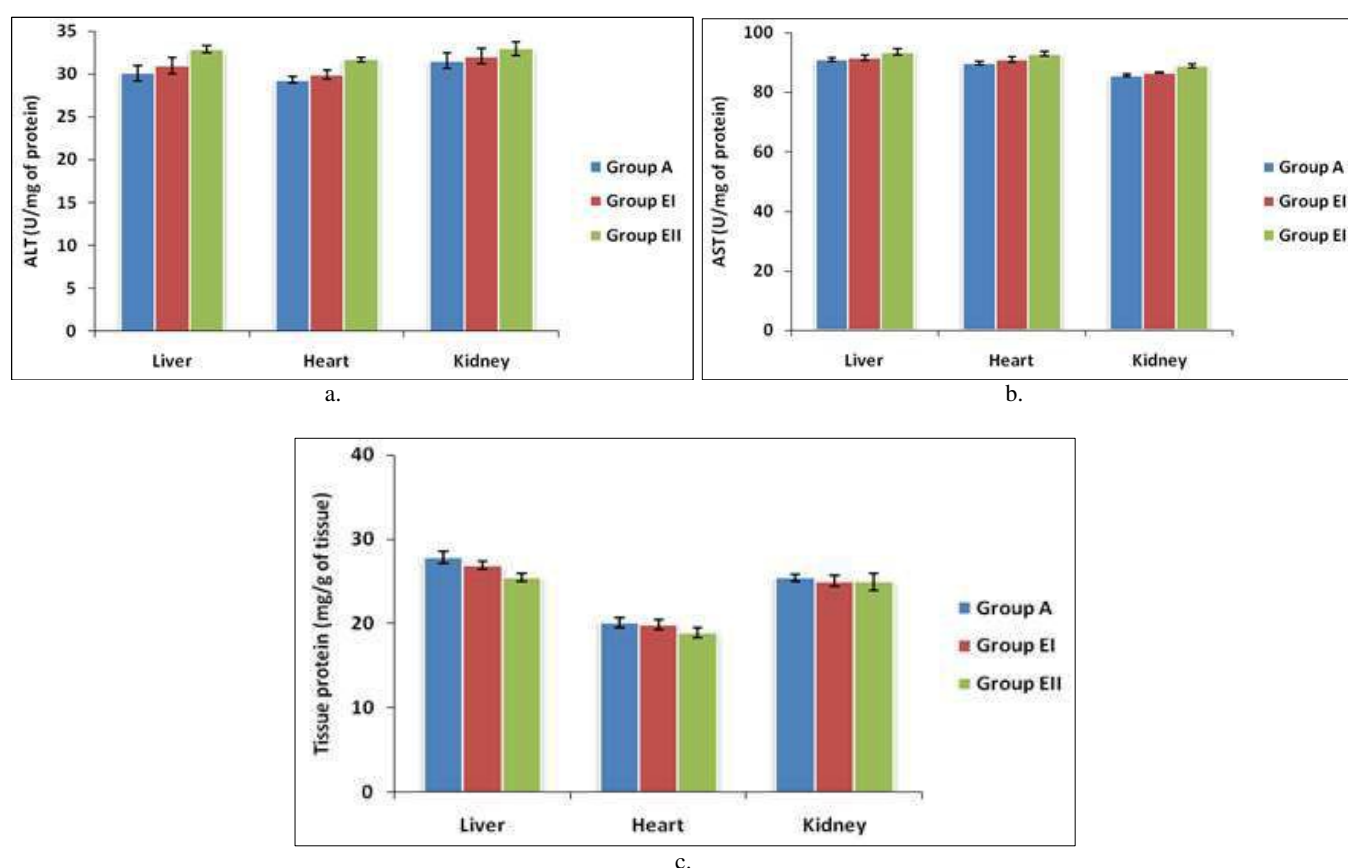


Fig 2 a, b, c: Effect of pretilachlor on tissue ALT (U/mg of protein), AST (U/mg of protein) and tissue protein (mg/g of tissue) values in goats following daily oral administration at two dose levels (152.5 and 305 mg/kg BW) for 28 days. (Mean $\pm$ SE of 4 replicates)

The effect of pretilachlor on lipid peroxidation was evaluated by measuring MDA levels in blood of goats, which increased significantly in group EII on days 14 and 28 compared to day 0 as depicted in figure 3a. This increase in MDA indicates lipid peroxidation in the plasma of goats, reflecting the peroxidation of polyunsaturated fatty acids in cell membranes resulting in cell damage. To counteract oxidative stress from lipid peroxidation and free radicals, the body's antioxidant defense mechanisms, including SOD, CAT, and GSH-PX, were upregulated. SOD activity increased significantly in groups EI and EII on day 28 as evident in figure 3b, while catalase activity also showed a significant increase in group EII on days 14 and 28 compared to day 0 as shown in figure 3c. These changes indicate a disruption in redox cycling processes and oxidative stress [31, 32]. Pretilachlor exposure also significantly increased MDA levels in liver tissue of goats in

group EII compared to groups A and EI (figure 4a). Whereas, kidney tissue showed a significant reduction in rGSH levels as shown in figure 4b, likely due to the kidneys' role in excreting xenobiotics and exhausting their thiol content to scavenge toxic free radicals. Lipoperoxidation was also higher in maternal and fetal livers of glyphosate-treated groups compared to controls, with the fetus showing greater differences due to lower antioxidant enzyme activity [33]. Blood GSH levels fluctuated markedly in pretilachlor-treated animals, as GSH is converted to GSSG to neutralize free radicals. The decrease in GSH after 28 days of exposure indicates depletion from counteracting pesticide-generated free radicals [34].

As represented in figure 4c, the cytochrome P450 activity significantly increased in group EII compared to group A. Similar results were observed with significant induction in hepatic P4502B enzyme activities and increased P4502B

apoprotein expression after administration of isoprothiolane, butachlor, or pretilachlor to male rats [35]. Glucose levels remained unaffected by pretilachlor exposure, contrary to findings in grass carps where 50% and 75% pretilachlor

exposure led to hyperglycemia. This hyperglycemia is an adrenaline or cortisol-mediated response to stress, providing tissues with energy [36].

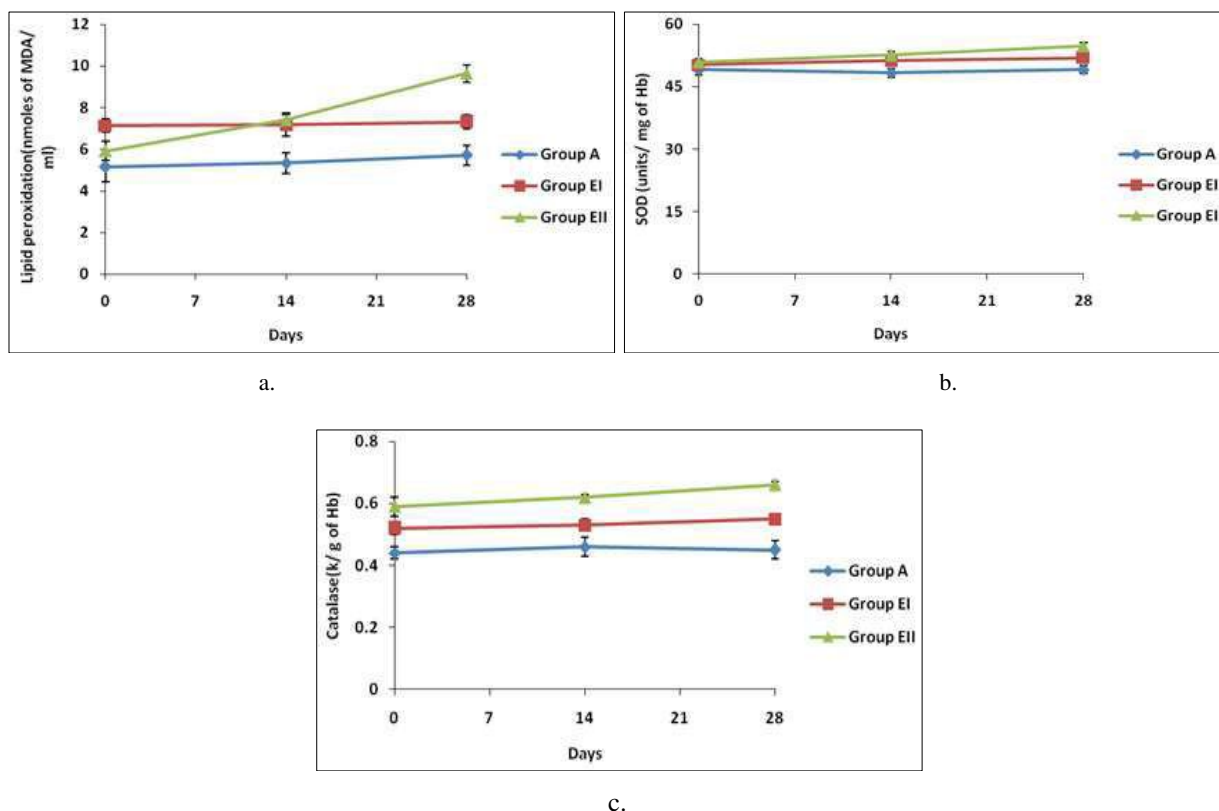


Fig 3 a, b, c: Effect of pretilachlor on plasma lipid peroxidation (nmol of MDA/ ml) blood SOD (units/ mg of Hb) and catalase enzyme level in goats following daily oral administration at two dose levels (152.5 and 305 mg/kg BW) for 28 days. (Mean±SE of 4 replicates)

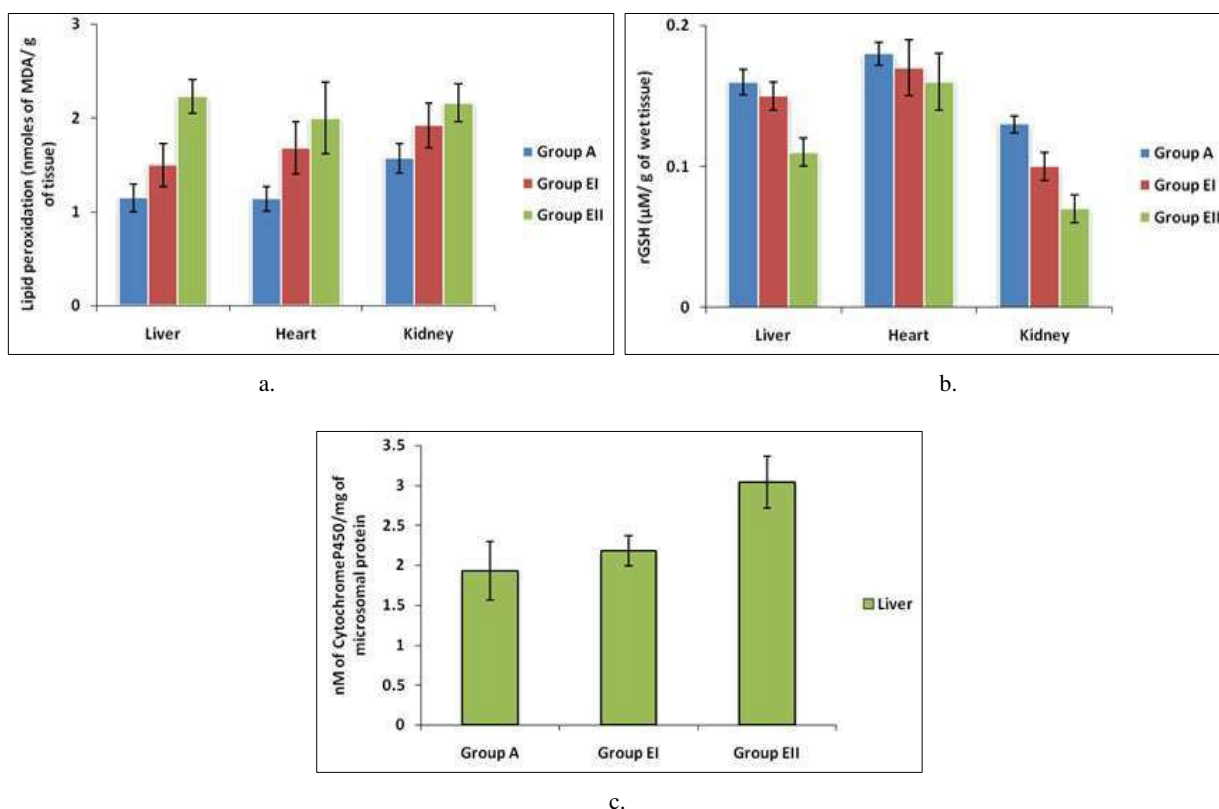


Fig 4a, b, c: Effect of pretilachlor on lipid peroxidation (nmol of MDA/ g of tissue), rGSH (μ M/ g of wet tissue) values and cytochrome P450 content in liver tissue of goats following daily oral administration at two dose levels (152.5 and 305 mg/kg BW) for 28 days. (Mean±SE of 4 replicates)

Analysis of pesticides

Standardization of recovery study of pretilachlor:

Chromatogram of standard and recovery of pretilachlor with retention time 5.62 min have been depicted in Figures 5 (a, b and c).

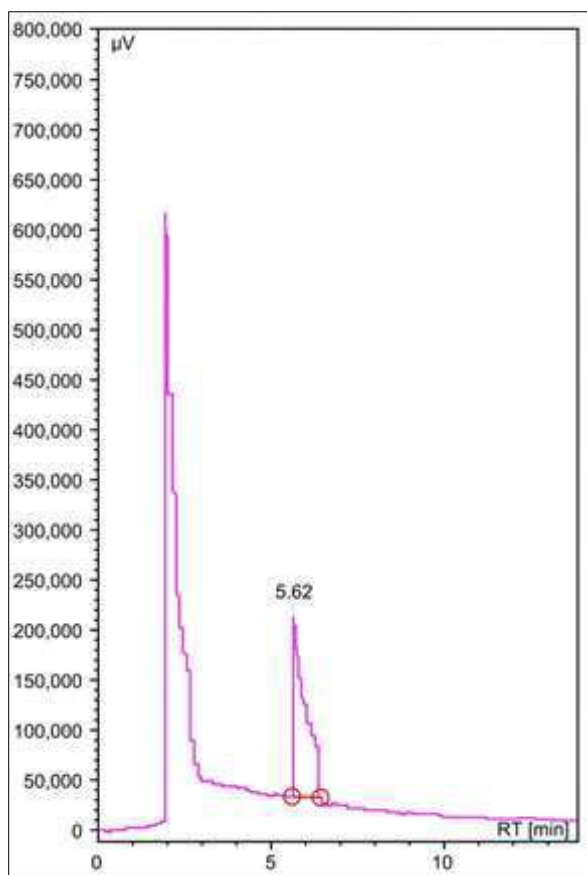


Fig 5(a): Chromatogram of pretilachlor (technical grade) 1ppm.

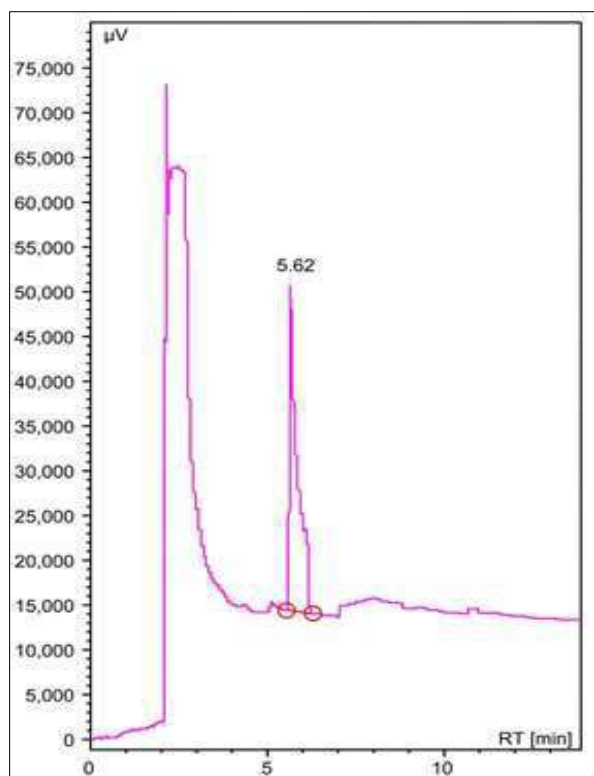


Fig 5(b): Chromatogram of pretilachlor extracted from blood

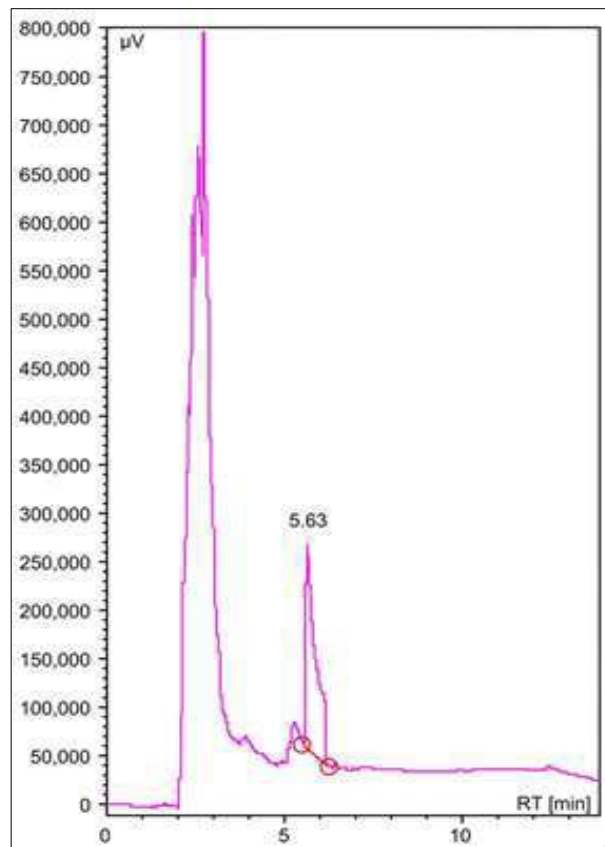


Fig 5(c): Chromatogram of pretilachlor extracted from liver.

The LOD and LOQ were found to be 0.12 and 0.42 ppm, respectively. The recovery study of pretilachlor from blood, bile and different tissues varied from 81-88%. Therefore, the method was found to be satisfactory and subsequently used for extraction, clean up and estimation of pretilachlor from blood, bile and tissues of goat.

Residues in the gastrointestinal tract, as evident from Table 4 were detected due to the oral administration of pretilachlor. Higher residue levels were found in the heart, lung, adrenal gland, muscle, fat, liver, and kidney, indicating greater accumulation in these organs. Residue detection in the spleen is likely due to pretilachlor binding with red blood cells, as supported by higher residue levels in the blood (Table 3). Biliary elimination is preferred over urinary elimination for phenyl-alkylated acetanilids, which aligns with our findings of higher residues in bile and liver [37]. Similar residue distribution was observed in freshwater fish *Channa punctata* exposed to butachlor, with residues in the order of liver > kidney > muscle > gill > brain. These variations are attributed to factors such as uptake rate, tissue lipid content, chemical structure, solubility, and metabolic patterns. Prolonged exposure to sublethal concentrations can increase residue accumulation [38].

Histopathology

Histological examination of liver tissue of goats in group EI revealed no pathological changes except hyperemia. In contrast, pyknosis, central vein dilation, congestion, and necrosis in the liver of Harness® (ACET)-treated *C. gariepinus* fish was reported²¹. Kidney tissue of goats in group EI showed fatty changes, hydropic degeneration of the lining epithelium, hyaline casts in the tubules, increased inter-tubular connective tissue, and mononuclear cell infiltration. Group EII goats exhibited similar changes, with

Table 3: Blood concentrations of pretilachlor (ppm) in goats were monitored at various time intervals and on different days following daily oral administration at two dose levels of 152.5 mg/kg BW and 305 mg/kg BW (Mean $\pm$ SE of 4 replicates).

Time in hour	Blood concentration (ppm)	
	Group EI (152.5mg/kg)	Group EII (305mg/kg)
1	BDL	BDL
2	BDL	BDL
3	BDL	1.37 $\pm$ 0.59
4	BDL	1.90 $\pm$ 0.71
6	3.83 $\pm$ 1.83	0.38 $\pm$ 0.09
8	2.44 $\pm$ 1.34	0.24 $\pm$ 0.08
12	0.58 $\pm$ 0.34	0.13 $\pm$ 0.04
16	BDL	BDL
20	BDL	BDL
24	BDL	BDL
36	BDL	BDL
48	BDL	BDL
72	BDL	BDL
7 th day	BDL	0.61 $\pm$ 0.27
14 th day	0.35 $\pm$ 0.14	4.61 $\pm$ 1.34
21 st day	0.55 $\pm$ 0.34	1.01 $\pm$ 0.59
28 th day	0.21 $\pm$ 0.08	1.03 $\pm$ 0.42

BDL: Below Detection Limit

Table 4: Pretilachlor concentrations (ppm) in various goat tissues were measured on day 29 and after a 15-day withdrawal period following daily oral administration at dose rates of 152.5 and 305 mg/kg BW for 28 days. (Mean $\pm$ SE of 4 replicates)

Tissues/ organs	Concentration (ppm)			
	Group EI (152.5 mg/kg)	After withdrawal period of 15 days	Group EII (305 mg/kg)	After withdrawal period of 15 days
Liver	0.20 $\pm$ 0.06	BDL	0.54 $\pm$ 0.14	BDL
Kidney	0.30 $\pm$ 0.07	BDL	0.43 $\pm$ 0.13	BDL
Heart	1.48 $\pm$ 0.49	BDL	1.83 $\pm$ 0.79	BDL
Lung	1.46 $\pm$ 0.40	BDL	3.06 $\pm$ 0.78	BDL
Muscle	0.74 $\pm$ 0.20	BDL	1.31 $\pm$ 0.42	BDL
Spleen	0.13 $\pm$ 0.05	BDL	0.21 $\pm$ 0.08	BDL
Fat	BDL	BDL	0.54 $\pm$ 0.19	BDL
Adrenal gland	1.14 $\pm$ 0.28	BDL	2.16 $\pm$ 0.44	BDL
Brain	0.30 $\pm$ 0.04	BDL	0.65 $\pm$ 0.10	BDL
Uterus	BDL	BDL	BDL	BDL
Bile	0.36 $\pm$ 0.30	BDL	1.03 $\pm$ 0.91	BDL
Skin	0.13 $\pm$ 0.08	BDL	0.38 $\pm$ 0.31	BDL
Large Intestine	0.77 $\pm$ 0.25	BDL	1.78 $\pm$ 0.60	BDL
Small Intestine	0.25 $\pm$ 0.08	BDL	0.58 $\pm$ 0.07	BDL
Rumen	0.15 $\pm$ 0.05	BDL	1.10 $\pm$ 0.49	BDL
Reticulum	0.15 $\pm$ 0.03	BDL	1.06 $\pm$ 0.52	BDL
Omasum	0.16 $\pm$ 0.05	BDL	0.85 $\pm$ 0.35	BDL
Abomasum	0.11 $\pm$ 0.02	BDL	0.47 $\pm$ 0.12	BDL
Bone	BDL	BDL	BDL	BDL
Mammary Gland	0.03 $\pm$ 0.01	BDL	0.12 $\pm$ 0.04	BDL
Urinary Bladder	0.13 $\pm$ 0.04	BDL	0.25 $\pm$ 0.08	BDL
Lymph Node	0.11 $\pm$ 0.04	BDL	0.23 $\pm$ 0.09	BDL

BDL: Below Detection Limit

additional blood vessel thickening due to medial hypertrophy, further inter-tubular connective tissue increased causing tubular atrophy, tubular epithelium proliferation, and progression to proliferative tubular nephritis. Renal histopathological changes like pyknosis, tubular degeneration, vacuolization, glomerulus shrinkage,

and Bowman's space dilation was also observed [21]. No changes were found in the heart of goats in group EI, while goats of group EII showed vessel congestion, hyperemia, muscle edema with separation, and mild Zenker's degeneration (Figure 6a). Similar cardiac alterations in *Clarias batrachus* exposed to butachlor was also recorded³⁴. In the spleen, no changes were observed in goats of group EI, whereas in group EII, there were reduced lymphoid follicles and lymphoid cell numbers. Lymph nodes in goats of group EI were unaffected, but group EII goats exhibited reduced lymphoid follicles and lymphocytes (Figure 6b).

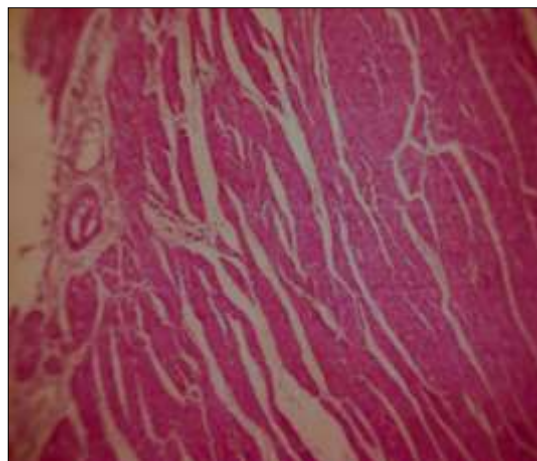


Fig 6a: Section of the heart showing mild Zenker's degeneration in the cardiac muscle of goats from group EII after daily oral administration of pretilachlor for 28 days. (H & E; 40X)

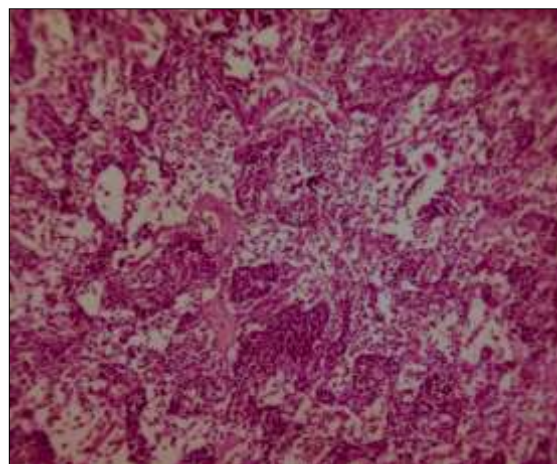


Fig 6b: Section of the lymph node showing a reduced number of lymphocytes in goats from group EII after daily oral administration of pretilachlor for 28 days. (H & E; 10X)

Conclusion

Pretilachlor [2-chloro-2, 6-diethyl-N-(propyloxyethyl) acetanilide] is a chloroacetanilide herbicide with moderate toxicity and significant adverse effects on aquatic organisms. Given the potential for systemic accumulation in food on exposure, we investigated the 28-day oral toxicity of pretilachlor in female black Bengal goats at doses of 305 mg/kg BW (minimum toxic dose) and 152.5 mg/kg BW (maximum non-toxic dose). No significant changes were observed in the hemogram of exposed animals. However, blood biochemical alterations, including increased serum AST and ALT levels, correlated with tissue biochemical changes and pathological alterations in kidney and heart tissue were recorded. Pretilachlor exposure induced the

cytochrome P450 enzyme system, indicating increased metabolic activity, and caused oxidative stress evidenced by elevated LPO in plasma and tissues, alongside increased blood antioxidant enzyme levels. Depletion of tissue glutathione levels suggested an active antioxidant defense mechanism. Tissue residues at the end of the experiment and after a withdrawal period indicated accumulation of pretilachlor in blood and tissues. Histopathological changes in liver, kidney, and lymph nodes were reversible, suggesting that pretilachlor had minimal toxic effects at the tested doses.

Conflict Of Interest: The authors declare no conflict of interest.

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