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Prophylactic Role of Piperine and Curcumin in Allethrin Altered Hematological and Biochemical Parameters in Swiss Albino Mice

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ABSTRACT

Background: Over the years, pyrethroids, including allethrin, are widely used for domestic and agricultural purposes and are found to be neurotoxic. However, effects on hematological and biochemical parametersare not elucidated. Thus, the first objective of the present study was to investigate the effect of allethrin on the hematological and biochemical parameters of Swiss albino mice. Further, for the amelioration of its effect, two different bioactive herbal extracts piperine (alkaloid) and curcumin (polyphenols) were evaluated. **Methods:** Animals were divided into six groups; the first group was used as a control. Groups 2, 3, 4, 5 and 6 were orally treated with allethrin (15 mg kg^{-1} b.wt.), allethrin+curcumin (100 mg kg^{-1} b.wt.), allethrin+piperine (100 mg kg^{-1} b.wt.), piperine (100 mg kg^{-1} b.wt.) and curcumin (100 mg kg^{-1} b.wt.), respectively for 28 days. **Results:** Administration of allethrin brought about a significant decrease in leukocytes, polymorphs, serum albumin, HDL, glucose and CPK and whereas, lymphocytes, haemoglobin, ALT, AST, serum creatinine, blood urea, total cholesterol, LDL, VLDL, triglycerides was found to be significantly increased following allethrin treatment. Gravimetric indices (body weight and organ weight) slightly declined following allethrin treatment. Further, the histopathological observations in allethrin treated mice showed the damage in all vital organs (kidney, liver, lungs, brain and heart) of Swiss albino mice. Administration of piperine and curcumin exhibited signi cant reversal of allethrin altered hematological and biochemical parameters. ALT, AST, serum creatinine, blood urea, serum albumin, HDL, glucose found to be comparable to that of the control group after piperine and curcumin administration. The presence of piperine and curcumin with allethrin preserved the normal histological architecture of the liver, kidney, lungs, brain and heart. **Conclusion:** These results indicate that piperine and curcumin can be a potent protective agent against allethrinalteredbiochemical and hematological alterations in mice.

Key words: Allethrin, hematological parameters, biochemical parameters, piperine, curcumin

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INTRODUCTION

Pyrethroidsare the most commoninsecticides used for agricultural and domestic purposes which account for 30% of insecticides used globally. The use of pyrethroids containing insecticidal products is increasing day by day due to their high insecticidal and low mammalian acute toxic effects[1-3]. Allethrin is a type I pyrethroid, one of the preferred household pest control insecticide having maximal human exposure as it is used as a main component in mosquito repellents[4,5]. Thus, there is a growing concern among the public regarding the routine and prolonged use of mosquito repellents.

In the literature, various reports have shown that allethrin induced toxicity on various organs and tissues of the mammalian system through apoptosis. It has been demonstrated that allethrin (125 µM) induced apoptosis in testicular carcinoma cells (LC_{450})[6]. The allethrin (5-100 µM) induced cell death in human corneal epithelial cells through mitochondrial dependent pathways of apoptosis[7].

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Allethrin (25 and 50 mg kg^{-1} b.wt.) induced genotoxicity in Swiss albino mice[8]. Thus, the management of its toxicity is important for the health of general populations.

Piperine ($C_{17}H_{19}NO_3$) is an alkaloid found in the fruits and roots of *Piper nigrum* and *Piper longum* species of Piperaceae family, with a long history of medicinal use in Indian medicine[9,10]. It exhibits a wide variety of biological effects, which includes; antimetastatic, antithyroid, antidepressant and hepatoprotective activity[11,12]. It has been evaluated the protective role of piperine (10 mg kg^{-1}, p.o.) against beryllium induced biochemical alteration and oxidative stress in female albino Wistarrats[13]. Recently, it has also been reported the cytoprotective role of piperinein deltamethrin induced thymocytes apoptosis[14].

Curcuma longa L. has been used for hundreds of years as a avor, color and preservative. Commercially, it is traded as a dye, spice and source of industrial starch[15]. Curcumin is a nutriceutical compound reported to possess a wide variety of biological activities including antioxidant[15], anticarcinogenic[16] and anti inflammatory activity[17]. Regarding its prophylactic role, it has shown that that curcumin (200 mg kg^{-1} b.wt., oral) plays a protective role in vancomycin induced nephrotoxicity by reducing oxidative stress[18]. Thus, these two herbals (piperine and curcumin) may play a major role in the attenuation of allethrin induced biochemical alterations.

Allethrin induced toxicity by apoptosis is well known, but regarding allethrin altered biochemical and haematological parameters, very few reports are available. Thus, the first objective of the current investigation is to explore the effect of allethrin on the hematological and biochemical parameters. Piperine and curcumin both are potent antioxidant, but their cytoprotective roles in the allethrin induced toxicity is still unexplored. Therefore, the second objective of the present study is to explore the role of piperine and curcumin in the modulation of biochemical and hematologicalparameters altered by allethrin.

MATERIALS AND METHODS

Chemicals: Allethrin, curcumin and piperine extract and all other chemicals were purchased from Sigma Aldrich, St. Louis, MO, USA. Leishman Stain Solution from Jupiter Reagents, Haematoxylin and Eosin dyes from Himedia, Cholesterol, Triglycerides, AST, ALT, creatinine, glucose and urea kits were purchased from Tulip Diagnostics (P) Ltd, India.

Instruments: Biochemistry Analyzer ARX-199i from Microlab Instruments, Ahmedabad, India.Incubator manufactured by Acme Instruments Co, Jaipur, India.

Dose selection: Doses were selected according to LD_{50} dose of allethrin for mice. Curcuminand piperinedose was selected according to previous studies performed by other researchers[19,20] and curcumin's pharmacological safety is accepted, considering that it has been consumed as a dietary spice, at doses up to 100 mg day^{-1}, for centuries[21]. The untreated control mice were treatedidentically with equal volumes of olive oil.

Animals and treatment: Male Swiss albino mice (4-6 weeks old), weight between 20-25 g, were selected for the study. They were housed in polypropylene cages with four of them in one cage. They were maintained at a temperature range of 22-24°C with access to standard animal food and clean drinking water. Our animal house and breeding facility register with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and CPCSEA guidelines were followed (IAEC approval obtained). Animals were divided into following six groups:

Group I : Received olive oil by oral gavage daily for 28 consecutive days and served as the control group

Group II : Received allethrin (25 mg kg^{-1}) for 28 days through oral route

Group III : Received curcumin (100 mg kg^{-1}) for 28 days through oral route

Group IV : Received piperine (100 mg kg^{-1}) for 28 days through oral route

Group V : Received curcumin (100 mg kg^{-1}) 1 h prior to allethrin (25 mg kg^{-1}) for 28 days through the oral route

Group VI : Received piperine (100 mg kg^{-1}) 1 h prior to allethrin (25 mg kg^{-1}) for 28 days through oral route

All the groups were treated for a 28 day period. At the end of each treatment,animals were weighed and sacrificed using light ether anesthesia. Blood samples for assay were collected under light ether anesthesia. Plasma samples were separated by centrifugation, frozen and stored at -20°C until assayed.

Hematological determination: Blood samples were collected in appropriate heparinized blood containers for determination of the Red Blood Cell (RBC), Leukocytes, Lymphocytes, platelets, hemoglobinusing Biochemistry Analyzer ARX-199i (Microlab Instruments).

Biochemical analysis: Total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, total cholesterol, triglyceride, HDL, LDL and VLDL were measured spectrophotometric ally in serum using Euro Diagnostickits by Biochemistry Analyzer ARX-199i (Microlab Instruments).

Histopathological studies: All the representative pieces of kidneys, liver, heart, lung and brainwere collected and xed in 10% formaldehyde followed by dehydration in graded alcohol and embedded in para n blocks. Fine sections were cut, mounted on glass slides and counter-stained with hema-toxylin-eosin (H and E) for light microscopic analyses.

Statistical analysis: Significance of the differences in the means (for each parameter) between the treatment groups was analysed using a one-way analysis of variance (ANOVA) after ascertaining the homogeneity of variance between the treatments. Pair-wise comparisons were performed by calculating the least signi cant difference. All data are reported as Mean±SD. All calculations were performed using Prism software (Graphpad, San Diego, CA). A p-value $p<0.001$, $p<0.01$, $p<0.05$ was accepted as indicative of significance.

RESULTS

Clinical observation: No deaths or remarkable signs of external toxicity were observed in the groups of mice that were given allethrin either alone or in combination with herbals. No overt sign of behavioral change was observed in the allethrin treated animals.

Body weight: Reduction in body weight was observed in allethrin treated mice after 28 days treatment. While, with mitigating agents (curcumin and piperine),showeda slight change in body weight (Table 1).

Organ/body weight ratio: The oragn/body weight ratio of all vital organs was increased in allethrin treated animals as shown in Table 2.

Hematological indices: The hematological parameters of allethrin treated mice were significantly altered. Further, both herbals (piperine and cucumin) pretreatment attenuated the allethrinaltered hematological parameters.

Erythrocytes count: The erythrocyte count was slightly increased in the allethrin treated group of animals as compared to control group but its non-significant increase. However, in the piperine alone group, there was a significant ($p>0.05$) decrease in erythrocytes count when compared with control (Fig. 1a).

Table 1: Average Initial weight and Final weight of all treatment groups of mice

		Average body weight (g)	
Groups	Dose	0 days	28 days
Control group	Olive oil	32.67	30.33
Allethrin group	25 mg kg^{-1}	27.33	22.33
Allethrin+curcumin group	25+100 mg kg^{-1}	28.33	28.33
Allethrin+piperine group	25+100 mg kg^{-1}	25.00	27.33
Curcumin group	100 mg kg^{-1}	30.67	29.67
Piperine group	100 mg kg^{-1}	30.33	27.67

Table 2: Percentage organs weight* of mice of all treatment groups after 28 days

Organs	Control group (wt. in %)	Allethrin group (wt. in %)	Allethrin+curcumin group (wt. in %)	Allethrin+piperine group (wt. in %)	Curcumin group (wt. in %)	Piperine group (wt. in %)
Kidney	2.626	3.239	2.505	1.988	2.483	1.879
Lungs	1.703	3.418	3.223	2.695	1.82	1.602
Liver	7.275	12.776	12.282	7.902	8.707	7.5
Heart	0.692	1.164	1.247	1.024	0.685	0.65
Brain	2.132	3.284	3.247	2.926	2.28	1.783

*Formula: ×100

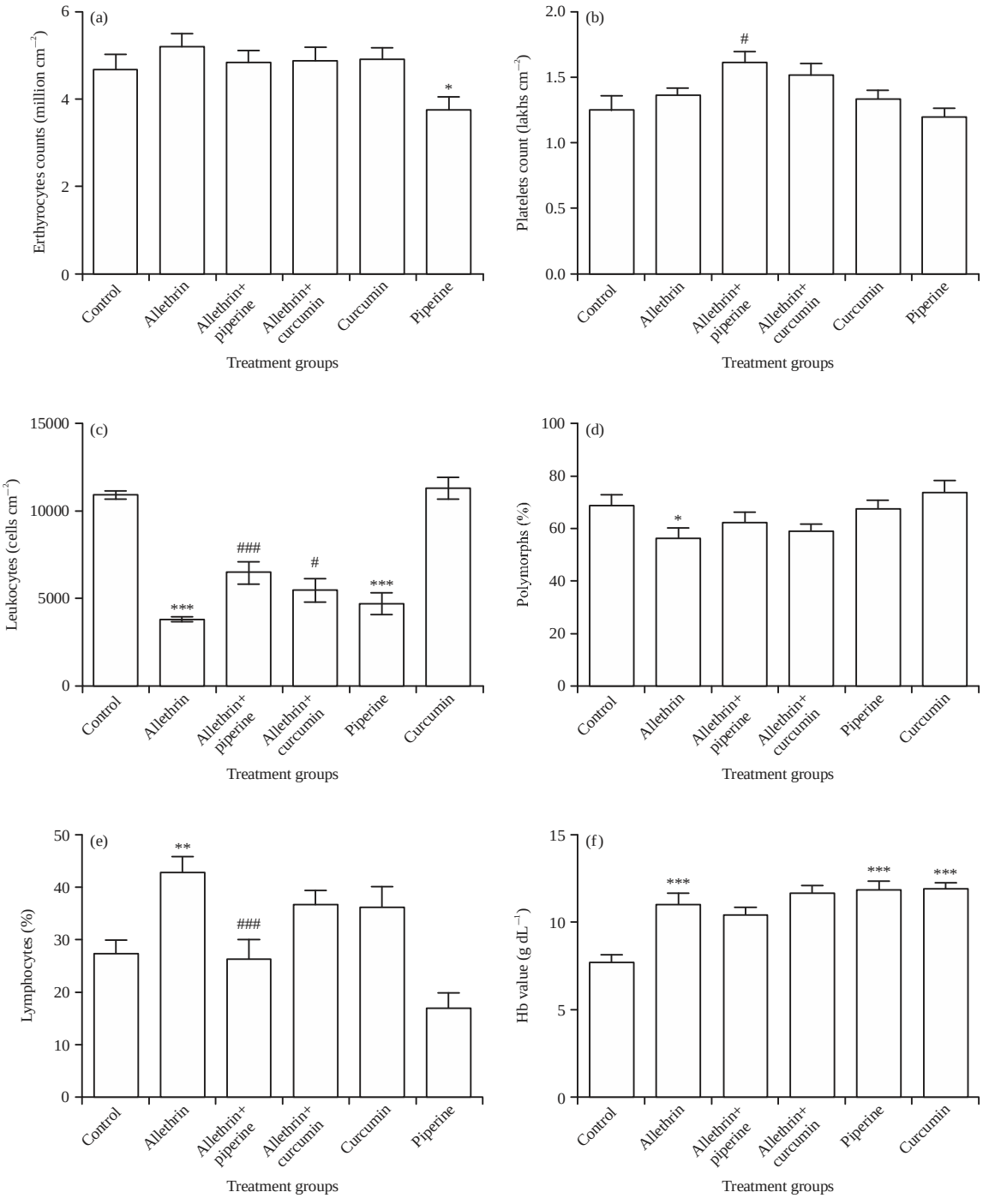

Fig. 1(a-f): Hematological parameters (a) Erythrocytes, (b) Platelets, (c) Leukocytes, (d) Polymorph (e) Lymphocytes and (f) Hemoglobin after allethrin, allethrin+piperine, allethrin+curcumin and piperine, curcumin alone treatment to mice for 28 days. Each bar represents Mean±SD (n = 6). Significantly different ***$p<0.001$, **$p<0.01$, *$p<0.05$ as compared to control group and number sign (#) means there are significant differences in comparison to allethrin treated group with allethrin+piperine and allethrin+curcumin treated group, using one-way ANOVA

Platelets count: There was a slight change in the platelet count of all experimental groups as compared to control, but all changes were non-significant ($p>0.05$) except allethrin+piperine group showed significant change ($p>0.05$), when exposed mice were compared to the control group (Fig. 1b).

Leukocytes: Allethrin treatment decreased the leukocytes counts significantly as compared with control groups (Fig. 1c). Both herbals (piperine and curcumin) supplementation along with allethrin increased the leukocytes count significantly, as compared to the allethrin group. Further, piperine alone also decreased the leukocyte count as compared to control. However, curcumin alone did not cause any significant change as shown in Fig. 1c.

Polymorphs: Allethrin treatment caused the significant decrease in the polymorphs count as compared to control group. Suppressed polymorphs were not significantly increased by piperine and curcumin. Further, piperine and curcumin alone did not cause any significant change as compared to control group (Fig. 1d).

Lymphocytes: Allethrin treatment caused a significant increase in the lymphocytes as compared to control as shown in Fig. 1e. Increased lymphocytes were significantly decreased by piperine, but curcumin treatment caused non-significant changes. Further, piperine alone decreased the lymphocytes count significantly as compared to control group (Fig. 1e).

Hemoglobin: Hemoglobin level was increased significantly ($p<0.001$) in allethrin treated group of animals. Both piperine and curcumin showed a non-significant change in hemoglobin level increased by allethrin. However, mice exposed to piperine and curcuminalone caused the significant ($p>0.001$) increase in hemoglobin when compared with control (Fig. 1f).

Biochemical assessments: Allethrin treatment caused the significant alteration in biochemical parameters, which are indicators of organs toxicity. Both piperine and curcumin, ameliorated the altered biochemical parameters.

Lipid profile: Both herbals attenuated the lipid profile altered by allethrin treatment.

High Density Lipoprotein (HDL): High Density Lipoprotein (HDL) is a good form of cholesterol, which is required for various biological activities. Allethrin caused significant decrease in the HDL level (Fig. 2a). The decreased level of HDL was significantly increased by the herbals (piperine and curcumin). Further, curcumin and piperine alone also caused a significant increase in the HDL level as compared to control.

Low density lipoprotein: Low Density Lipoprotein (LDL) is a bad form of cholesterol, whose increased level affects the normal biological activities. The significant increase in LDL was observed in the allethrin treated group. Piperine pretreatment caused significant decrease in the LDL level, which was increased by allethrin (Fig. 2b). Curcumin treatment did not cause any significant change.

Very low density lipoprotein: Allethrin caused a significant increase in the VLDL level. Both herbals (piperine and curcumin) caused a slight decrease in the VLDL level, but in a non-significant manner (Fig. 2c). Further, piperine and curcumin alone did not cause any significant change in VLDL level as compared to control.

Triglycerides: Allethrin induced triglyceride level was not attenuated by the herbals (piperine and curcumin). However, both piperine and curcumin alone caused a significant increase in the triglyceride level (Fig. 2d).

Total cholesterol: The level of total cholesterol was significantly increased in the allethrin treated group of animals as compared to control group. A Significant decrease was observed when the animals were pretreated with the piperine (Fig. 2e). However, curcumin pretreated animals did not show any significant change.

Hepatic markers

Alanine amino transferase: Alanine amino transferase (ALT) is an indicator of hepatic function. In allethrin treated group, significant elevation in ALT was observed. Further, the elevated ALT level was significantly reduced by piperine and curcumin (Fig. 3a). Both piperine and curcuminalone did not cause any significant change.

Aspartate amino transferase: Allethrin caused a significant increase in the AST level. This increased level

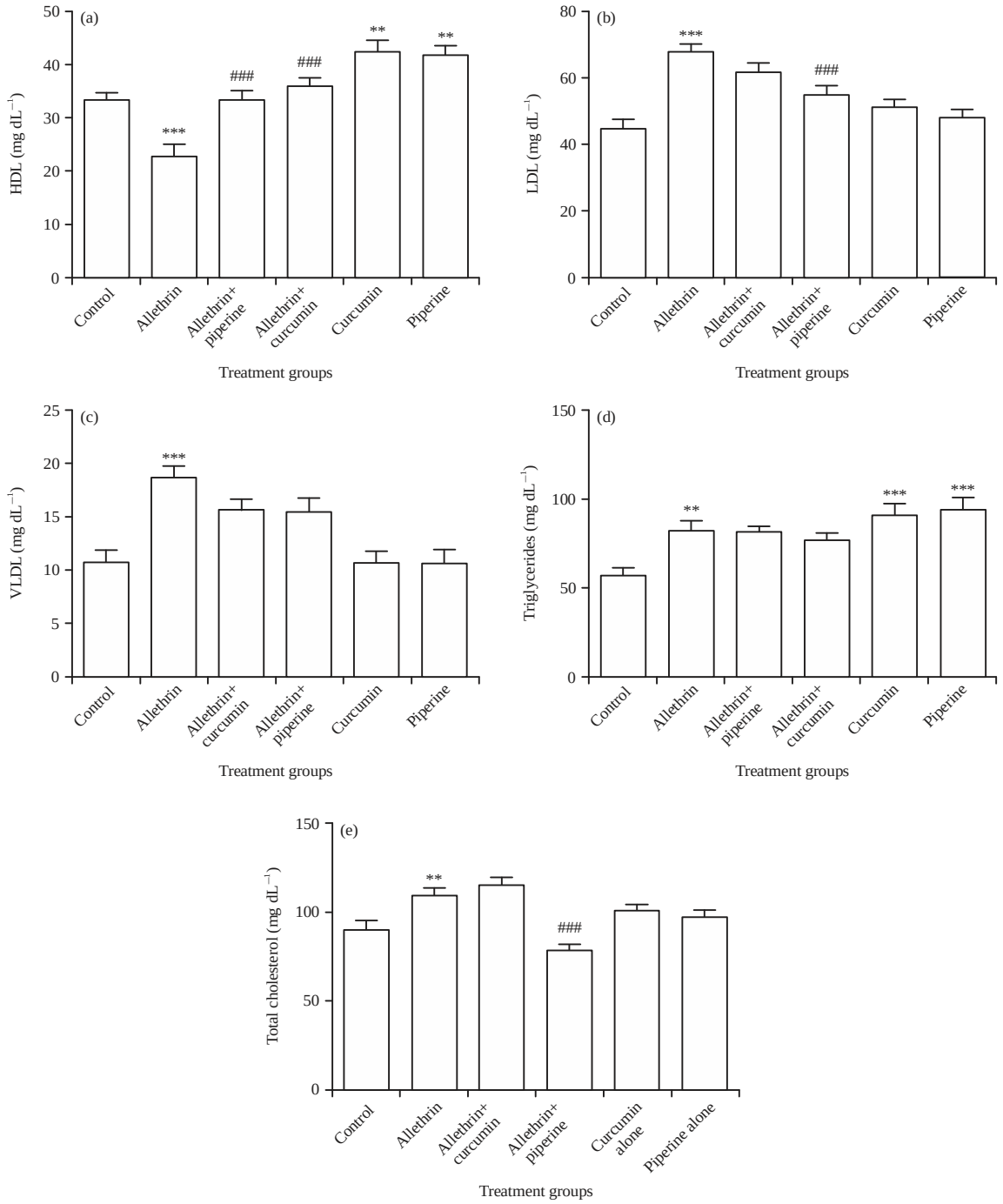

Fig. 2(a-e): Total lipid profile, (a), HDL, (b) LDL, (c) VLDL, (d) Triglycerides and (e) total cholesterol after allethrin, allethrin+piperine, allethrin+curcumin and piperine, curcumin alone treatment to mice for 28 days. Each bar represents Mean±SD (n = 6). Significantly different ***p<0.001, **p<0.01, *p<0.05 as compared to control group and number sign (#) means there are significant differences in comparison to allethrin treated group with allethrin+piperine and allethrin+curcumin treated group, using one-way ANOVA

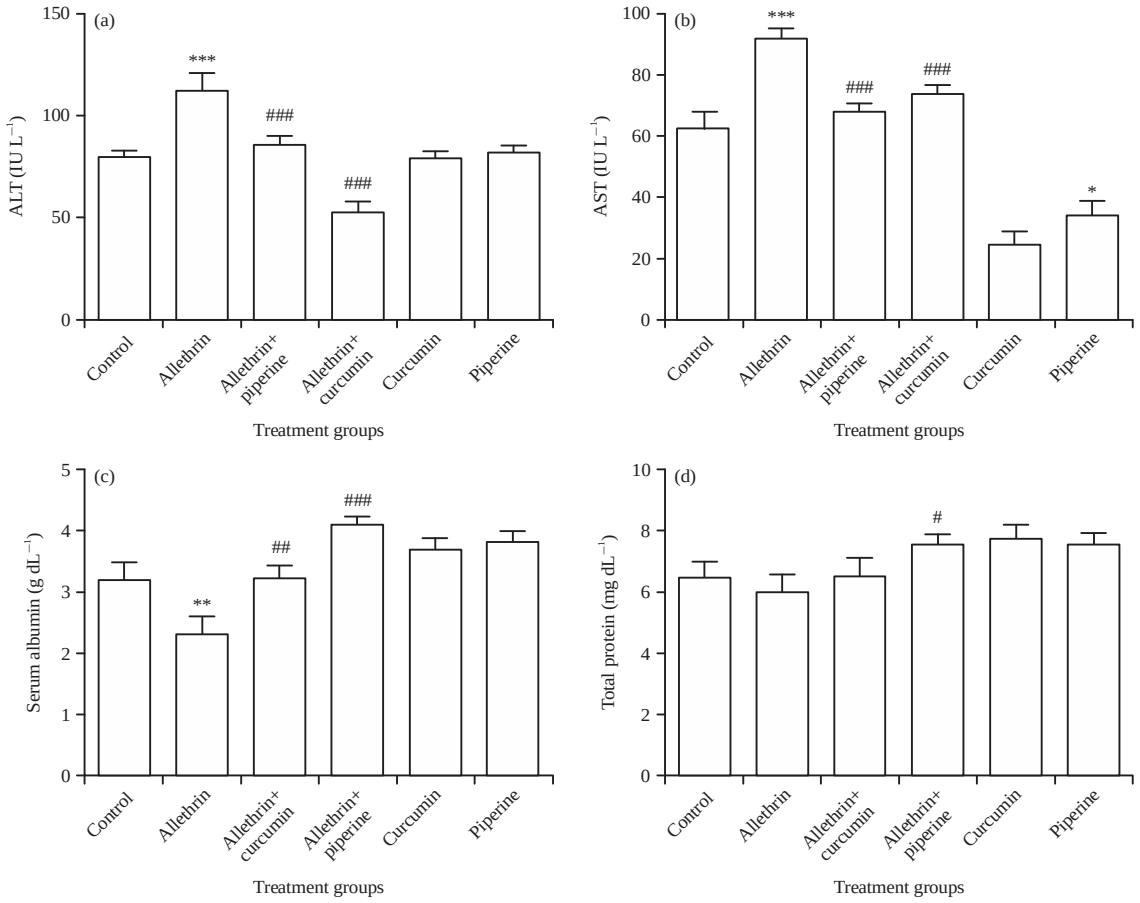

Fig. 3(a-d): Hepatic markers, (a) ALT, (b) AST, (c) Serum albumin and (d) Total protein pro le after allethrin, allethrin+piperine, allethrin+curcumin and piperine, curcumin alone treatment to mice for 28 days. Each bar represents Mean±SD (n = 6). Significantly different ***p<0.001, **p<0.01, *p<0.05, as compared to control group and number sign (#) means there are significant differences in comparison to allethrin treated group with allethrin+piperine and allethrin+curcumin treated group, using one-way ANOVA

of AST was significantly ameliorated by herbals (piperine and curcumin). However, piperine alone also caused a significant increase in the AST level (Fig. 3b).

Albumin: Allethrin caused a significant decrease in the serum albumin level as compared to control group animals. The decreased level of albumin was significantly increased by the piperine and curcumin (Fig. 3c). Further, both herbals (piperine and curcumin) alone did not cause any significant change.

Total protein: The total protein level was slightly decreased in the allethrin treated group of animals but in non-significant manner. After treatment with herbals, piperine showed significant increase in the protein level as compared to the allethrin group of animals (Fig. 3d). Both herbals alone did not cause any significant alteration.

Renal markers

Serum creatinine: Allethrin caused a significant increase in the creatinine level as compared to control group of animals. Both curcumin and piperine pretreatment significantly decreased the creatinine level increased by allethrin (Fig. 4a). The both herbals alone did not cause any significant change in serum creatinine level as compared to control group.

Blood urea: Blood urea was significantly increased in the allethrin treated group of animals. Both piperine and

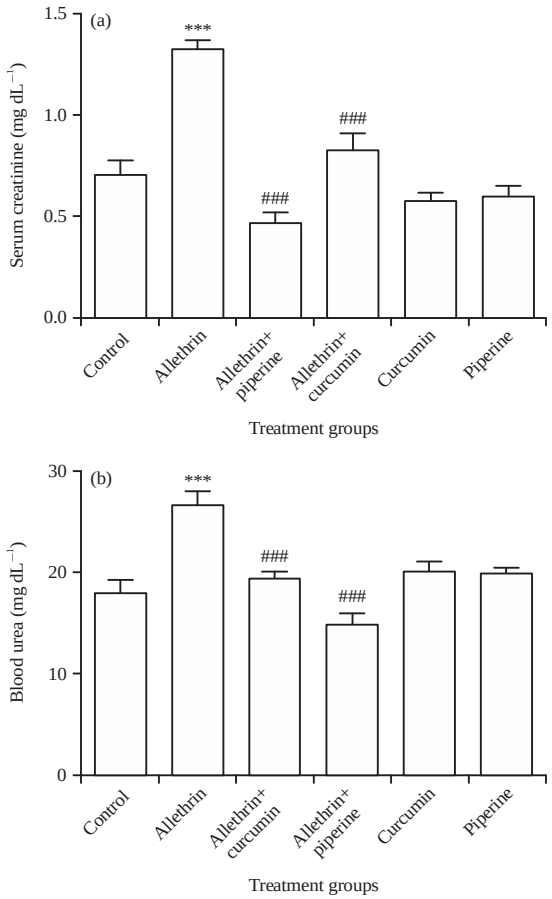

Fig. 4(a-b): Renal markers, (a) Serum creatinine and (b) Blood urea profile after allethrin, allethrin+piperine, allethrin+curcumin and piperine, curcumin alone treatment to mice for 28 days. Each bar represents Mean±SD (n = 6). Significantly different ***p<0.001, **p<0.01, *p<0.05 as compared to control group and number sign (#) means there are significant differences in comparison to allethrin treated group with allethrin+piperine and allethrin+curcumin treated group, using one-way ANOVA

curcumin showed significant decrease in the blood urea level as compared to the allethrin group of animals (Fig. 4b). Further, both herbals alone did not cause any significant alteration.

Glucose: Allethrin caused significant decrease in the glucose level as compared to the normal group of animals. The reduced level of glucose was significantly

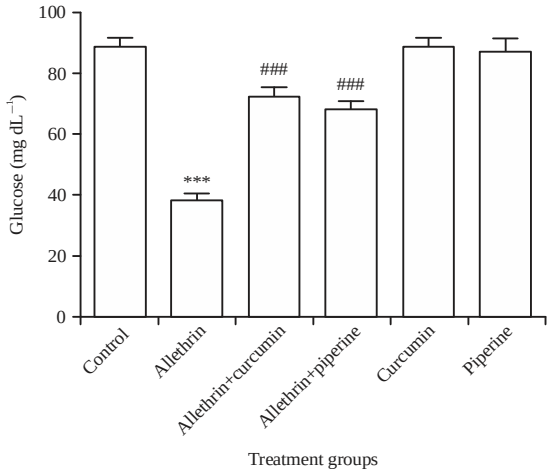

Fig. 5: Glucose level after allethrin, allethrin+piperine, allethrin+curcumin and piperine, curcumin alone treatment to mice for 28 days. Each bar represents Mean±SD (n = 6). Significantly different ***p<0.001, **p<0.01, *p<0.05 as compared to control group and number sign (#) means there are significant differences in comparison to allethrin treated group with allethrin+piperine and allethrin+curcumin treated group, using one-way ANOVA

increased by the both herbals as shown in Fig. 5. The alone groups of both herbals did not show any significant alteration as compared to control.

CPK: The CPK level was significantly increased by the allethrin. Both herbals pretreatment were not able to decrease its level significantly (Fig. 6). The alone groups of both herbals did not show any significant alteration in CPK level as compared to control.

Histopathological analyses

Kidney: Allethrin treated mice revealed a consistent feature of degenerative, infiltrative and vascular changes (Fig. 7). Degenerative changes indicated by the continued progression from reversible to irreversible stages of cellular injury, which includes; cellular swelling along with marked granular and vascular changes in majority of lining epithelial cells of proximal convoluted tubules, but such changes were milder in distal convoluted as well as medullary tubules. Irreversibly injured cells, revealing the features of coagulative necrosis characterized by nuclear swelling, picnosis Karyorrhexis, karyolysisalong with cytomegaly,

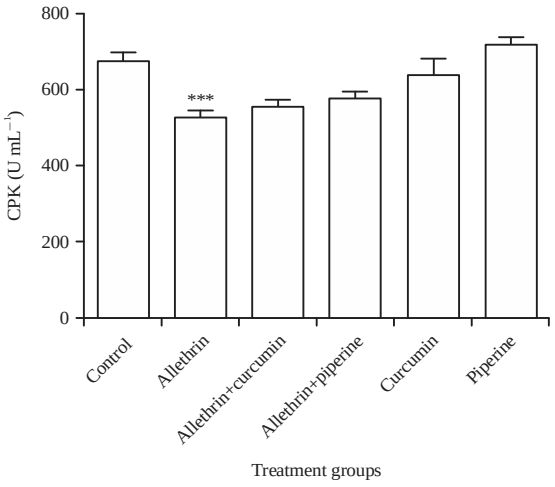

Fig. 6: CPK pro le after allethrin, allethrin+piperine, allethrin+curcumin and piperine, curcumin alone treatment to mice for 28 days. Each bar represents Mean±SD (n = 6). Significantly different ***p<0.001, **p<0.01, *p<0.05 as compared to control group and number sign (#) means there are significant differences in comparison to allethrin treated group with allethrin+piperine and allethrin+curcumin treated group, using one-way ANOVA

individualization and cytoplasmolysis of lining epithelial cells in Proximal Convoluted Tubules (PCT). There were circulatory disturbances marked by congestion of intertubular blood vessels along with interstitial edema. Multifocal mononuclear cell infiltration was also evident in the interstitial spaces in some reason.

Both piperine and curcumin treated mice alongwith allethrin showed less damage to kidney, as compared to allethrin alone treated mice. However, milder degenerative, infiltrative and vascular changeswereobservedin these groups of animals (Fig. 7). Further, piperine and curcumin alone treated mice failed to reveal any pathological changes of significance except mild congestion.

Liver: Microscopic section of liver of allethrin treated mice showed degenerative changes in hepatocytes progressing from reversible to irreversible stages of cellular injury. Reversible changes were characterised by cellular swelling alongwith granular and vascular changes of hepatocytes, more pronounced in periportal area than in centrilobular zone (Fig. 8). Irreversibly injured cells, revealing the feature of coagulative necrosis as evident by karyorrhexis, picnosis, karyolysis and anucleosis along with cytoplasmolysis, individualisation and disintegration of hepatocyes. This leads to atrophy of hepatic cords preserving the architecture of hepatic cord and hepatic lobules. However, in some focal areas due to more marked coagulative necrosis there was a loss of lobular architecture also (Fig. 8). Moreover mononuclear cells infiltration and proliferation of bile duct in portal areas were also observed.

In the piperine and curcumin treated mice along with allethrin treatment, showed protective effect against allethrin induced damage in the liver. However, some degenerative, infilterative and vascular changes were also observed but less as compared to allethrin alone treated animals. In both herbals alone groups, no significance change was observed.

Lungs: Microscopic section of the lung of allethrintreated mice showed mild congestion of blood vessels with focal microscopic hemorrhages. At some places peribronchiolar lymphocytes were hyperplastic along withthe presence of perivascular cuffing (Fig. 9). There were occasional presences of emphysematous changes also.

Both piperine and curcumin showed the protective effect against allethrin induced degenerative changes in the lungs. Further, curcumin and piperinealone showed no change of significance as compared to control groups of animals.

Brain: Microscopic examination of allethrin treated mice showed mild congestion of blood vessel, neuronal edema and satellitosis alongwith occasional neuronal degeneration. These all degenerative changes were protected by piperine and curcumin (Fig. 10).

Heart: Microscopic section of the heart of allethrin treated mice revealed mild granular to vacuolar changes with some of isolated myofibrils showing hyalization and changes of Zenkers necrosis. Moreover congestion of blood vessels alongwith focal haemorrhages and mononuclear cell infiltration was also observed. However, such degenerative changes were comparatively milder in curcumin and piperine treated mice. Contrary to it no change of significance was observed in controlled, curcumin and piperine alone treated mice (Fig. 11).

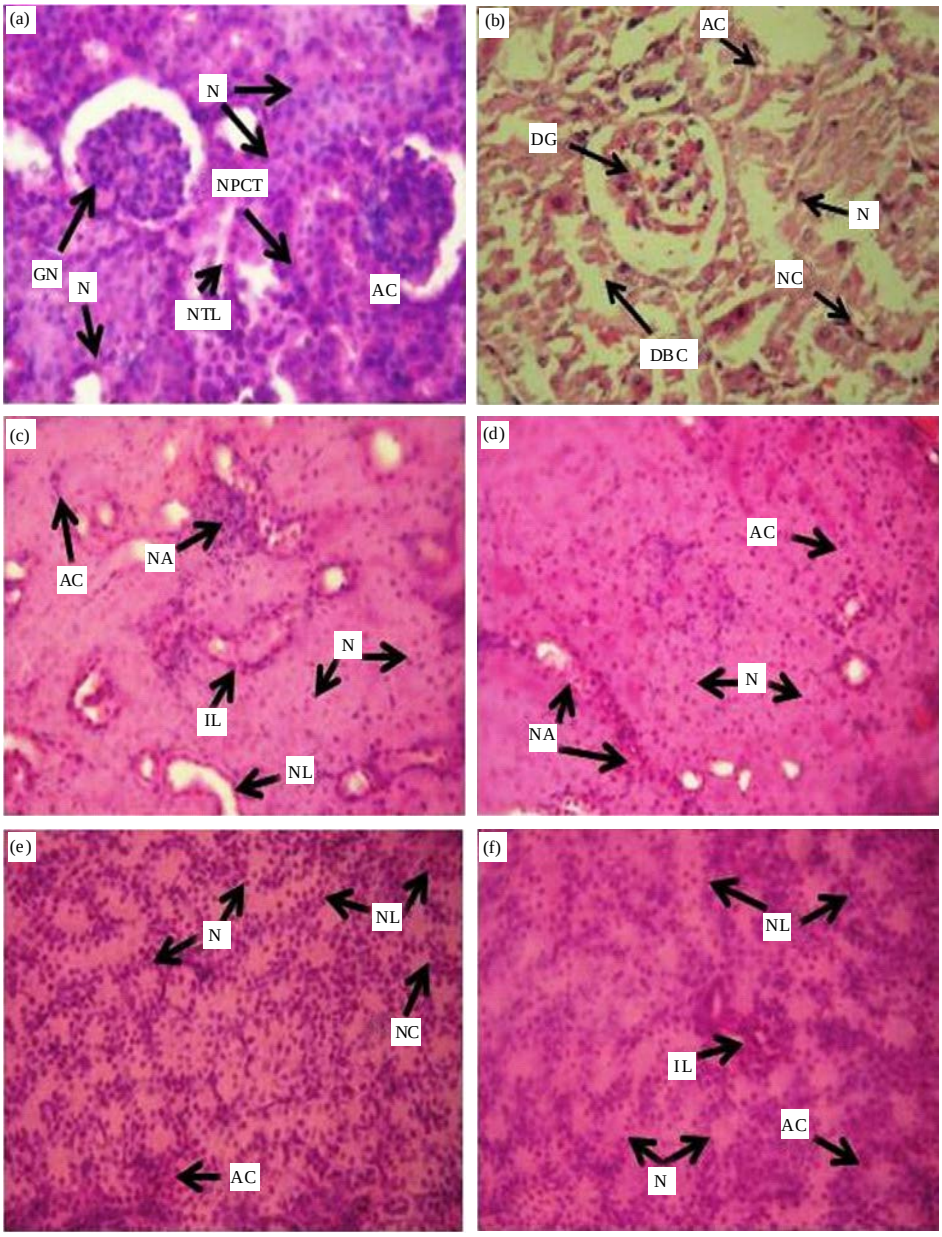

Fig. 7(a-f): Microscopic evaluation of kidney of all treatment groups, (a) Control group, (b) Allethrin group, (c) Allethrin+curcumin group, (d) Allethrin+piperine group, (e) Piperine group and (f) Curcmingroup. N: Normal nuclei, GN: Normal glomerulus, NC: Necrotic cells, AC: Apoptotic cells, DG: Damaged glomerulus, DBC: Damaged Bowman's capsule, NA: Necrotic area, NPCT: Normal proximal convoluted tubules, NL: Normal lining of tubules, IL: Inflammed lining of tubules

DISCUSSION

Allethrin is a non-cyano-containing a pyrethroid insecticide used extensively for controlling flies and mosquitoes. Overnight use of allethrin-based mosquito repellents is an extremely common practice in many households worldwide with delivery systems ranging from liquid vaporizers, mats, coils to sticks[22]. This suggests unavoidable exposure to many populations and therefore, it is essential to evaluate its toxic potential along with management of its toxicity.

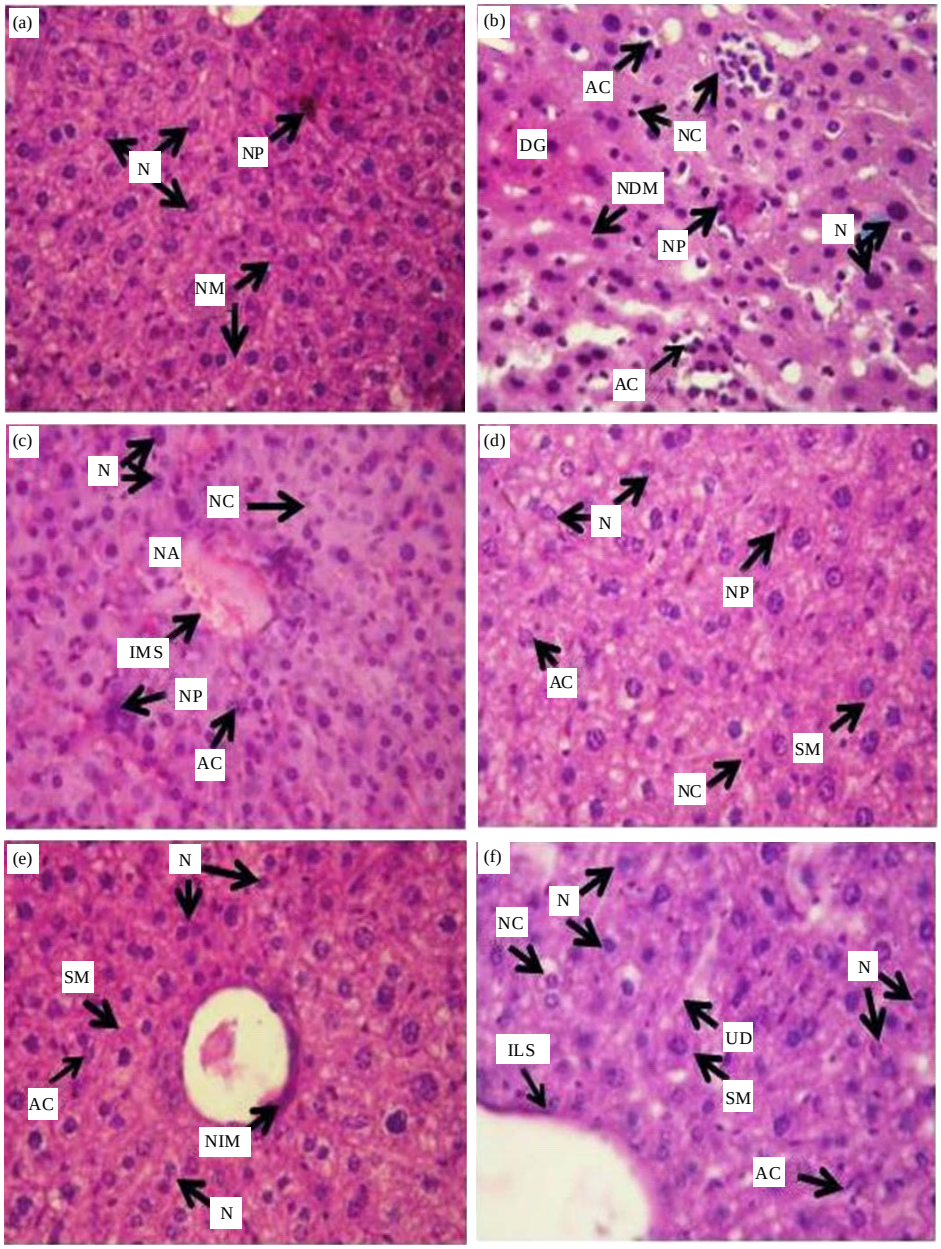

Fig. 8(a-f): Microscopic evaluation of liver of all treatment groups, (a) Control group, (b) Allethrin group, (c) Allethrin+curcumin group, (d) allethrin+piperine group, (e) Piperine group and (f) Curcmin group. N: Normal nuclei, NP: Necrotic parts, NM: Normal cellular membrane, SM: Swelled cellular membrane, NDM: No defined membrane of cell found, NC: Necrotic cells, AC: Apoptotic cells, NP: Necrotic parts, NIM: Normal inner membrane of blood vessels, ILS: Inner line swelling of blood vessels

The present study demonstrates the protective potential of curcumin and piperine by reversing the allethrin altered hematological and biochemical parameters. In the allethrin treated group, slight decrease in body weight was observed at the end of the experimental period. It may be due to the effect of allethrin on gastrointestinal tract, which results in decreased appetite and absorption of nutrients from the

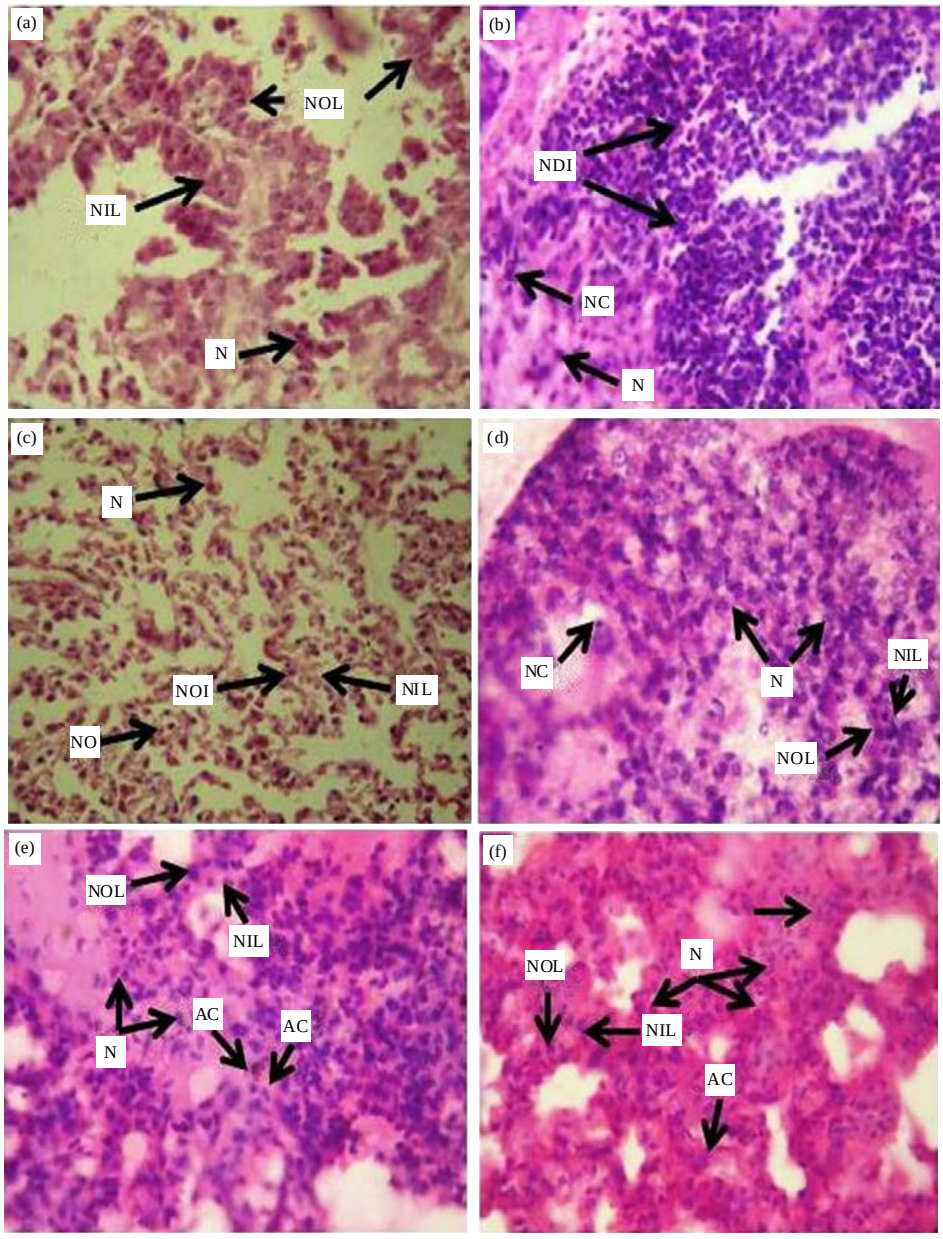

Fig. 9(a-f): Microscopic evaluation of lungs of all treatment groups, (a) Control group, (b) Allethrin group, (c) Allethrin+curcumin group, (d) Allethrin+piperine group, (e) Piperine group and (f) Curcmin group. N: Normal nucleus, NIL: Normal inner lining of alveoli, NOL: Normal outer lining of alveoli, NDL: No defined inner and outer lining, NC: Necrotic cell

gut or might be due to direct toxicity of allethrin. The results of the present study revealed that allethrin causes significant alteration in the hematological and biochemical parameters.

The toxicity of any exogenous compound can be assessed by evaluation of the hematological parameters. The toxic effect of the compound is usually seen, as an increase or decrease of blood cells and excess or deficit of one or more serum markers[23,24]. In the present investigation, RBC, WBC, hemoglobin and platelets had been measured. There was a non-significant change in RBC and platelet count in the allethrin treated group as

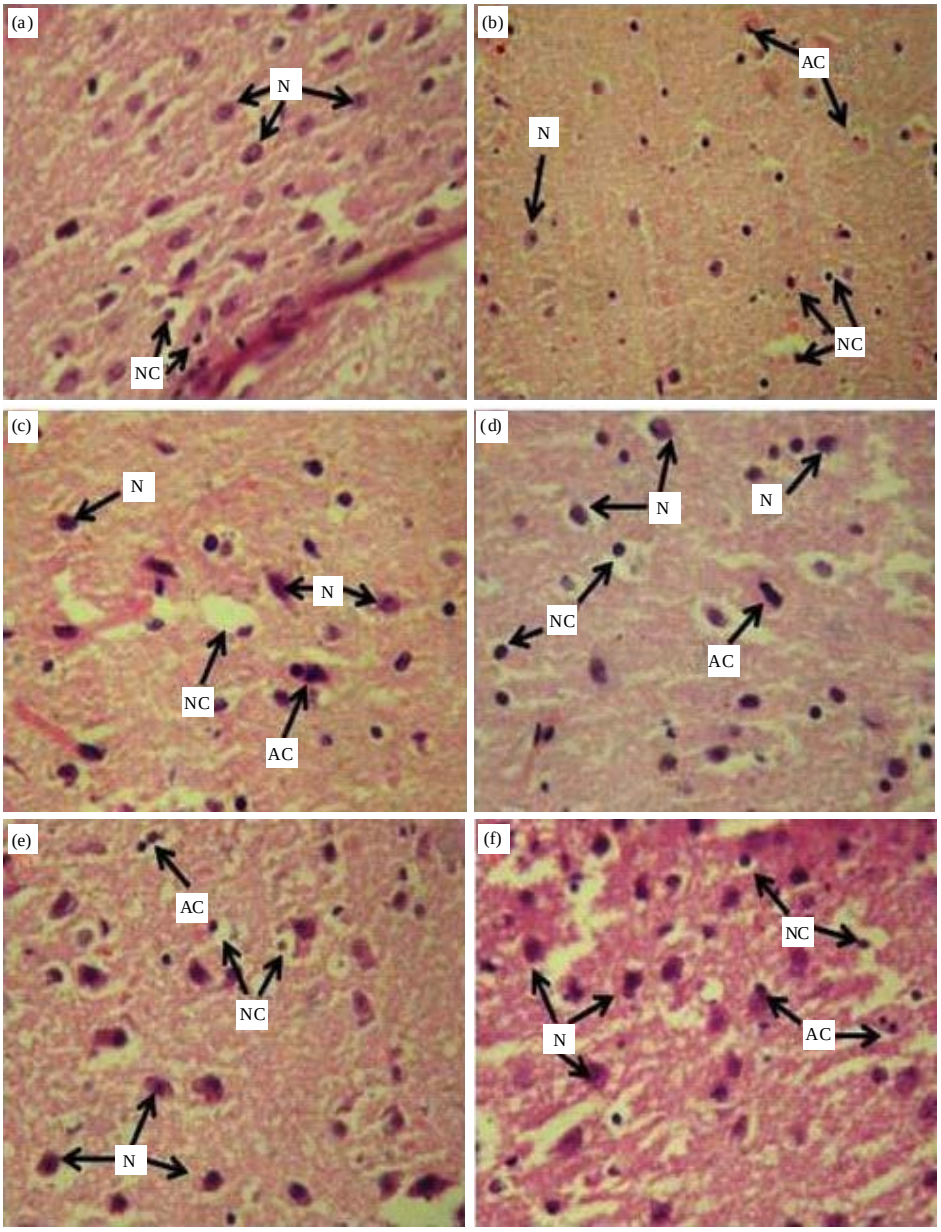

Fig. 10(a-f): Microscopic evaluation of brains of all treatment groups, (a) Control group, (b) Allethrin group, (c) allethrin+curcumin group, (d) Allethrin+piperine group, (e) Piperine group and (f) Curcmin group. N: Normal nuclei, NC: Necrotic cells, AC: Apoptotic cells

compared to control. However, leukocytes have been significantly decreased and lymphocytes have significantly increased in the allethrin treated group. Thus, it can be concluded that hematological parameters observed do not suggest anemia as a risk factor in allethrin exposure. However, marked increase in lymphocytes recorded in mice exposed to allethrin indicates its carcinogenic potential. Glucose is the main source of energy. There was a significant decrease in the glucose level of the allethrin treated group of animals.

Total protein, albumin, ALT and AST are some indicators of hepatic function[25]. Both AST and ALT were increased in allethrin treated animals. The

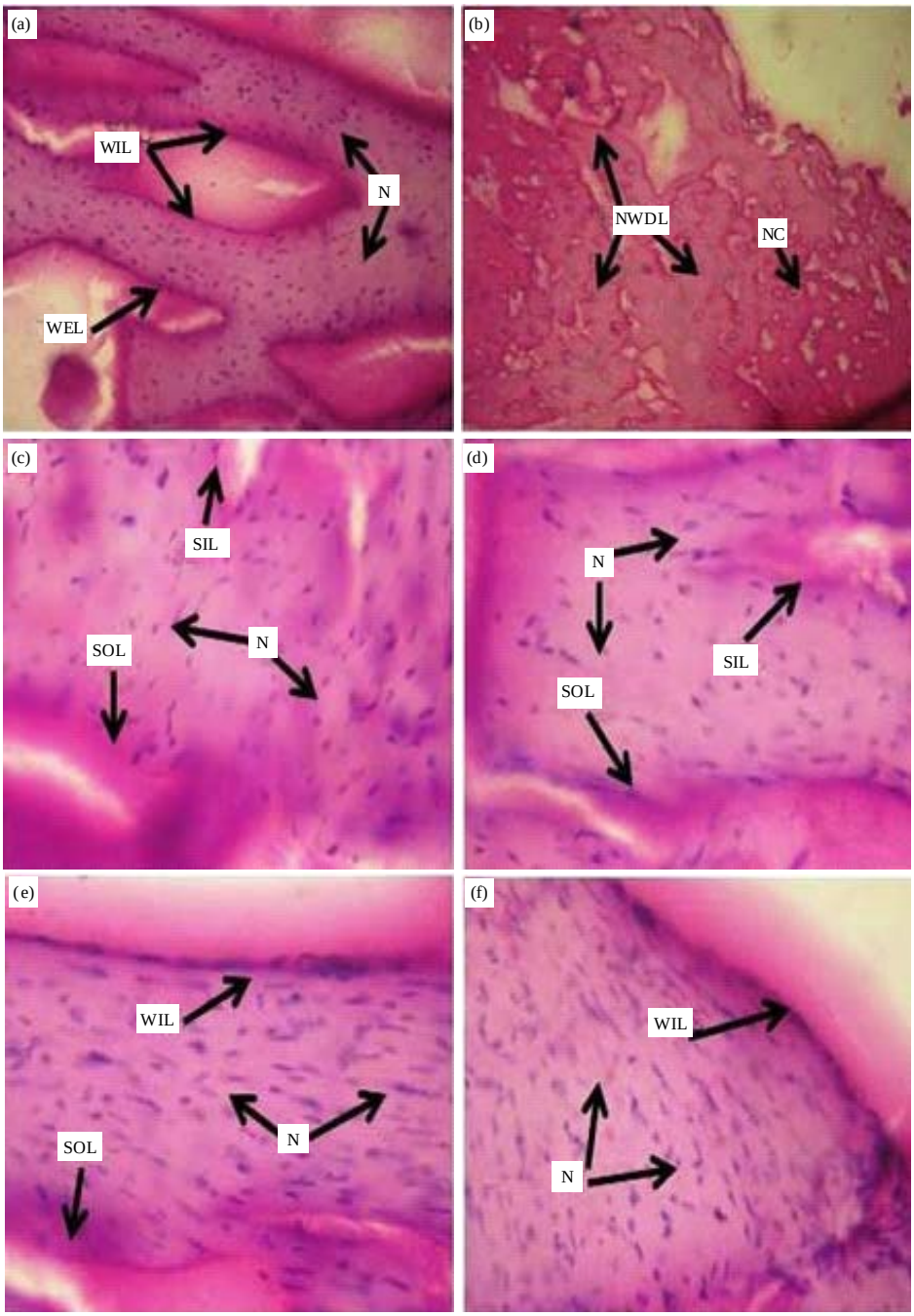

Fig. 11(a-f): Microscopic evaluation of heart of all treatment groups, (a) Control group, (b) Allethrin group, (c) Allethrin+curcumin group, (d) Allethrin+piperine group, (e) Piperine group and (f) Curcmin group. N: Normal nuclei, SOL: Swelled outer lining of cells, SIL: Swelled inner lining of cells NWDL: No well-defined lining of cells, WEL: Well defined outer lining, WIL: Well defined inner lining, NC: Necrotic cells

increased levels of AST and ALT in allethrin treated group could be due to cellular injury of the liver tissues. It has also been reported that the pyrethroid induced liver toxicity by increasing the activities of AST and

ALT[26]. In this study, at the end of 28 days, no change was observed in the total protein level. Albumin is synthesized by the liver. Albumin most often transports or binds drugs or chemical. Albumin level was decreased after allethrin treatment as compared to the control group. The histopathological observations in allethrin treated mice showed degenerative changes in hepatocytes characterized by cellular swelling along with granular and vascular changes, more pronounced in periportal area than in the centrilobular zone (Fig. 8). This study, therefore demonstrates the potential damage to the liver arising from the use of allethrin.

Serum creatinine and Blood Urea Nitrogen (BUN) are considered as significant markers of renal function. The present study indicated that treatment with allethrin caused a significant increase in serum creatinine and BUN, demonstrating the alteration of the renal function, in comparison with those of the control. This was also confirmed by histopathological examination of mice exposed to allethrin, 28 days in this study, which indicates damage in the lining of epithelial cells of Proximal Convoluted Tubules (PCT). Similar changes due to pyrethroid insecticide exposure have been also observed in rabbits[26].

Pyrethroid insecticides generally caused an increase of total cholesterol and total lipid levels. Allethrin caused an increase in cholesterol level of mice. Pesticides induced increase in serum cholesterol can be attributed to the effect of pesticides on the permeability of the liver cell membrane[27]. Also, the increase in serum total cholesterol level may be attributed to the blockage of the liver bile ducts causing a reduction or cessation of its secretion to the duodenum[28]. In present study, allethrin caused decrease in HDL and increase in the LDL, triglycerides, total cholesterol and VLDL-cholesterol level.

Various herbals around the world are used as cytoprotective agents. Piperine and curcumin are two well-known herbals, which act as a cytoprotective[14,18,29,30] but the exact role in allethrin induced toxicity is still unclear. Animals that received curcumin and piperine along with allethrin showed a slight increase in body weight as compared to allethrin alone treated mice, which indicate that these both herbals have appetite inducer and antistress effect. The results of present investigation have shown that piperine and curcumin treatment prevents the elevation of serum biochemical enzymes and histopathological alteration. Further, both piperine and curcumin alone did not cause any toxicity.

CONCLUSION

In conclusion, this study indicates that curcumin and piperine have the protective effect against allethrin altered biochemical and hematological parameters of mice. In view of the earlier reports on the toxic potential of allethrin in laboratory experiments and from the present study, further studies are needed to assess the possible risks to human health caused by allethrin. For attenuation of its toxic effects, our present data suggest that oral treatment with piperine and curcumin supplementation, significantly decreases the toxicity of allethrinbut their mechanism of protection is unclear so far. Thus, their mechanism of protection in allethrin induced toxicity needs to be further investigated.

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