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Histopathological changes by combined exposure of cypermethrin and deltamethrin and their amelioration by *Withania somnifera* and resveratrol in rats

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Abstract
Cypermethrin and deltamethrin are synthetic pyrethroid insecticide used for pest control in agriculture and as an acaricide in man and animals. The combined exposure of cypermethrin and deltamethrin was given in adult male Wistar rats and their amelioration by *Withania somnifera* and resveratrol on histology of liver and kidney was investigated upto 14 and 28 days. The combined treatment of cypermethrin and deltamethrin caused significant changes in liver *viz.* congestion, mild perivascular reaction, mild fatty change and sinusoidal dilation and in kidney *viz.* hypercellularity in glomeruli and hyaline degenerative change in parietal layer of glomerulus in 14 days study while focal area of necrosis of hepatocytes with mild infiltration of mononuclear cells in liver and focal interstitial nephritis with congestion and infiltration of mononuclear cells in kidney in 28 days study. In the cypermethrin plus deltamethrin plus *Withania somnifera* group and cypermethrin plus deltamethrin plus resveratrol group, both *Withania somnifera* and resveratrol co-treatment restored the changes to normal observed following combined cypermethrin and deltamethrin exposure in rats. The present study showed that combined cypermethrin and deltamethrin exposure caused histopathological changes which is reversed and restored to normal following co-treatment with *Withania somnifera* and resveratrol. This indicates the ameliorating effects in rats exposed to cypermethrin and deltamethrin.

Keywords: histopathology, liver, kidney, cypermethrin, deltamethrin, resveratrol and *Withania somnifera*

1. Introduction
Synthetic pyrethroids is a unique group of insecticides having pyrethrin like structure with better performance characterized and account for over 30% of insecticide use globally. Pyrethroids are modified derivatives of pyrethrins, natural substance obtained from flowers of *Pyrethrum* species. Pyrethroids are widely used in agriculture and veterinary applications due to their high bioefficacy, enhanced stability and comparatively low mammalian toxicity.

Cypermethrin is a synthetic pyrethroid insecticide which is used to kill insects especially on cotton. It behaves as a fast-acting neurotoxin in insects. Cypermethrin is used in agriculture to control ectoparasites which infest cattle, sheep and poultry [2]. In veterinary medicine, it is effective in controlling ticks on dogs. Synthetic pyrethroids affect axons of neurons of peripheral nervous system and central nervous system. It interacts with transportation system of sodium ions through cellular membrane. This results a delay in closing of sodium channel and a prolonged sodium tail current after membrane gets repolarized. Thus cypermethrin acts as neurotoxic for both insects and mammals [3].

Deltamethrin is a synthetic pyrethroid insecticide used in agriculture, home pest control and disease vector control. Neurotoxic mechanisms of deltamethrin include, prolonging the opening of voltage sensitive sodium channels and inhibition of voltage gated chloride channels and GABA receptors [15].

Resveratrol is a fat soluble compound that occurs in *trans* and *cis* configuration. Resveratrol (trans-3,5,4-trihydroxystilbene), a polyphenolic phytoalexin abundantly found in grapes and red wine is a potent antioxidant and cytoprotective agent. Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a stilbenoid, a type of natural phenol, and a phytoalexin produced naturally by several plants in response to injury or when the plant is under attack by pathogens such as bacteria or fungi [6]. The major dietary sources of stilbenes include grapes, wine, soy, peanuts, and peanuts products [4]. *Pediomelum cuspidatum* root has been used for long in traditional asian medicine, as a circulatory tonic, is a source of resveratrol [6].

Withania somnifera is a plant in the Solanaceae or nightshade family. It is used as a herb in ayurvedic medicine. The main chemical constituents are alkaloids and steroidal lactones.

Withania somnifera possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoetic, and rejuvenating properties [12]. *Withania somnifera* is a well-known and important medicinal plant widely used in several indigenous system of medicine for treatment of various ailments *viz*. asthma, inflammatory disease, bronchitis, ulcer and stomach problems. Major Phyto constituents of this species are steroidal lactones. Pharmacological experiments in a number of *in-vitro* and *in-vivo* models have demonstrated the ability of *Withania somnifera* to exhibit anti-inflammatory, antiulcer, antidiabetic, central nervous system depressants and hepatoprotective activities leading support to the rationale behind several of its traditional uses [9].

2. Materials and Methods

2.1 Drugs and chemicals

Cypermethrin and deltamethrin formulations were purchased from Bayer Crop Science Ltd., India. Resveratrol was procured from Sigma-Aldrich Company. Methanolic extract of *Withania somnifera* were prepared in the departmental laboratory.

2.2 Animals and treatment

A total of 84 adult male Wistar rats weighing 100-120 g were procured from Disease Free Small Animal House (DFSAH), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, and housed in polyacrylic cages in a group of 7 rats per cage in the departmental animal house. Bedding material (rice husk) was changed on alternate days. The animals were provided with feed and water *ad libitum* and maintained at room temperature with a natural light-dark cycle. Rats were acclimatized to laboratory conditions for 7 days before the start of experiment. Animal house temperature varied between 22 to 27º C throughout the study. The prior approval of institutional animal ethics committee was obtained for the use of experimental animals in this study. Forty two rats were used for 14 days study, while remaining forty two rats were used for 28 days study. The rats were randomly divided into six groups, each comprising of seven rats. Group 1 was Naive (control) group which received 3% gum acacia suspension orally. Group 2 was given cypermethrin (75 mg/kg) plus deltamethrin (4 mg/kg) as suspension in 3% gum acacia orally. Group 3 animals received cypermethrin (75 mg/kg) plus deltamethrin (4 mg/kg) as suspension in 3% gum acacia and separately *Withania somnifera* (12.5 mg/kg) suspension in 3% gum acacia orally. Group 4 animals were administered cypermethrin (75mg/kg) plus deltamethrin (4 mg/kg) as suspension in 3% gum acacia and separately resveratrol (5 mg/kg) suspension in 3% gum acacia orally. Group 5*Withania somnifera* (12.5 mg/kg) in 3% gum acacia suspension was administered orally. Group 6 Resveratrol (5 mg/kg) in 3% gum acacia suspension was administered orally. Experiment groups were same for 14 days as well as for 28 days study.

2.3 Sampling

Animals were sacrificed under ether anesthesia 12 hours after last administration of cypermethrin and deltamethrin. Just after sacrificing, vital organs *viz*. liver and kidney were excised free from surrounding tissues and weighed. Tissues were put in 10% buffered formalin for subsequent processing and histopathological studies. The formalin fixed tissues were thoroughly washed in running tap water, dehydrated in ascending grades of alcohol, cleared in benzene and embedded in paraffin. 5 µ thick sections from paraffin embedded tissues were stained by haematoxylin and eosin (H and E) method. The sections were examined for the pathological findings of hepatic and renal changes.

2.4 Statistical Analysis

Data were expressed as mean ± SE. Statistical analysis of data was performed using Graph Pad prism 5.03 and Microsoft Excel. Data were analyzed by ANOVA along with Bonferroni multiple comparison post hoc test. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 In 14 days study

3.1.1 Liver

Histopathological lesions in the liver of naive and other treatment groups are presented in Figure 1. Histopathological investigations demonstrated that combined cypermethrin and deltamethrin treated group have mild degenerative changes in hepatic tissue *viz*. congestion, telangiactasis (sinusoidal dilation), mild fatty changes in hepatocytes and mild perivascular reaction. *Withania somnifera* co-treatment in combined cypermethrin and deltamethrin treatment group showed improvement in histopathological changes of liver by diminishing congestion, telangiactasis (sinusoidal dilation) and perivascular reaction. Resveratrol co-treatment in combined cypermethrin and deltamethrin treatment groups showed improvement in histopathological changes of liver by diminishing congestion and perivascular reaction. In *Withania somnifera* and resveratrol alone treated groups, there was no alteration found in histological architecture of liver and hepatocytes.

3.1.2 Kidney

Histopathological lesions in the kidney of naive and other treatment groups are presented in Figure 2. Histopathological investigations demonstrated that naive, *Withania somnifera* and resveratrol alone treated group have intact glomeruli and renal tubules in the cortical area of kidneys whereas combined cypermethrin and deltamethrin treated groups have mild degenerative changes *viz.* hypercellularity in glomeruli and hyaline degenerative change in parietal layer of glomerulus with mild infilterationof MNCs in interstitium. *Withania somnifera* co-treatment and Resveratrol co-treatment in combined cypermethrin and deltamethrin treatment group showed improvement in histopathological changes of kidney by diminishing the hypercellularity in glomeruli and hyaline degenerative change in glomerulus.

3.2 In 28 days study

3.2.1 Liver

Histopathological lesions in the liver of naive and other treatment groups are presented in Figure 3. Histopathological investigations demonstrated that naive, *Withania somnifera* and resveratrol group have normal hepatocytes arranged in cord pattern around the central vein whereas combined cypermethrin and deltamethrin treated groups have moderate degenerative changes *viz.* focal necrosis of hepatocytes with mild infilteration of MNCs and mild reaction in portal area. *Withania somnifera* and resveratrol co-treatment in combined cypermethrin and deltamethrin treated groups showed improvement in histopathological changes of liver by diminishing degeneration of hepatocytes and reaction in portal area.

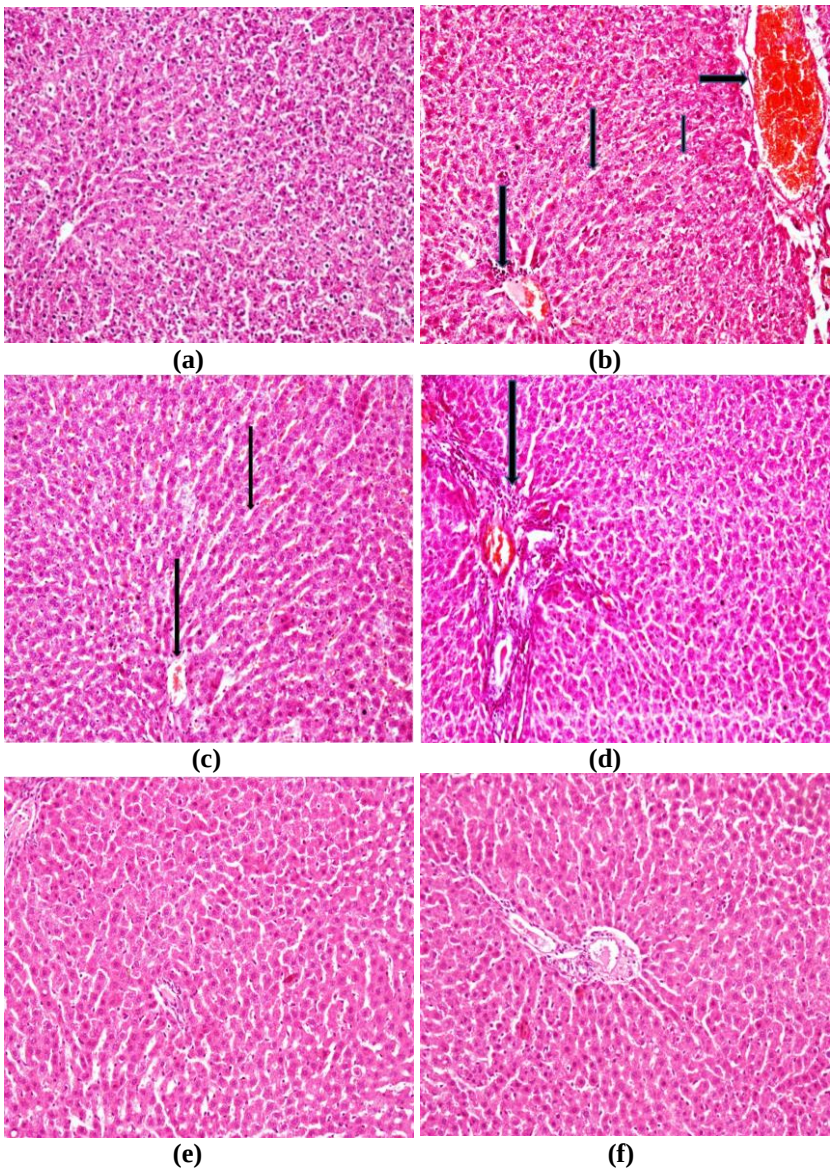
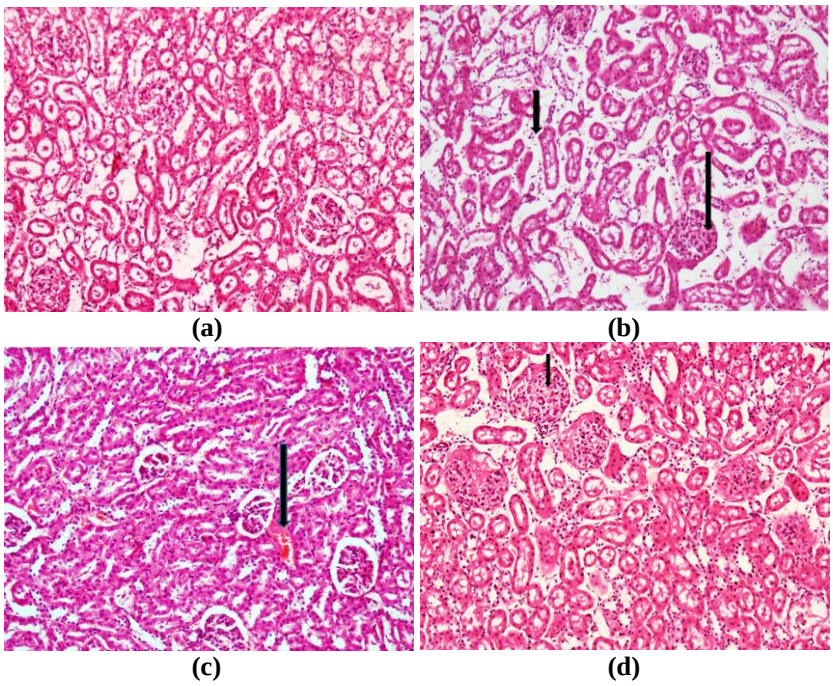
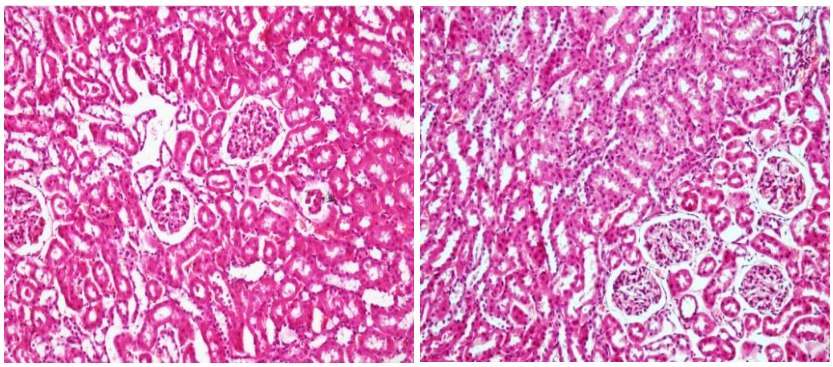


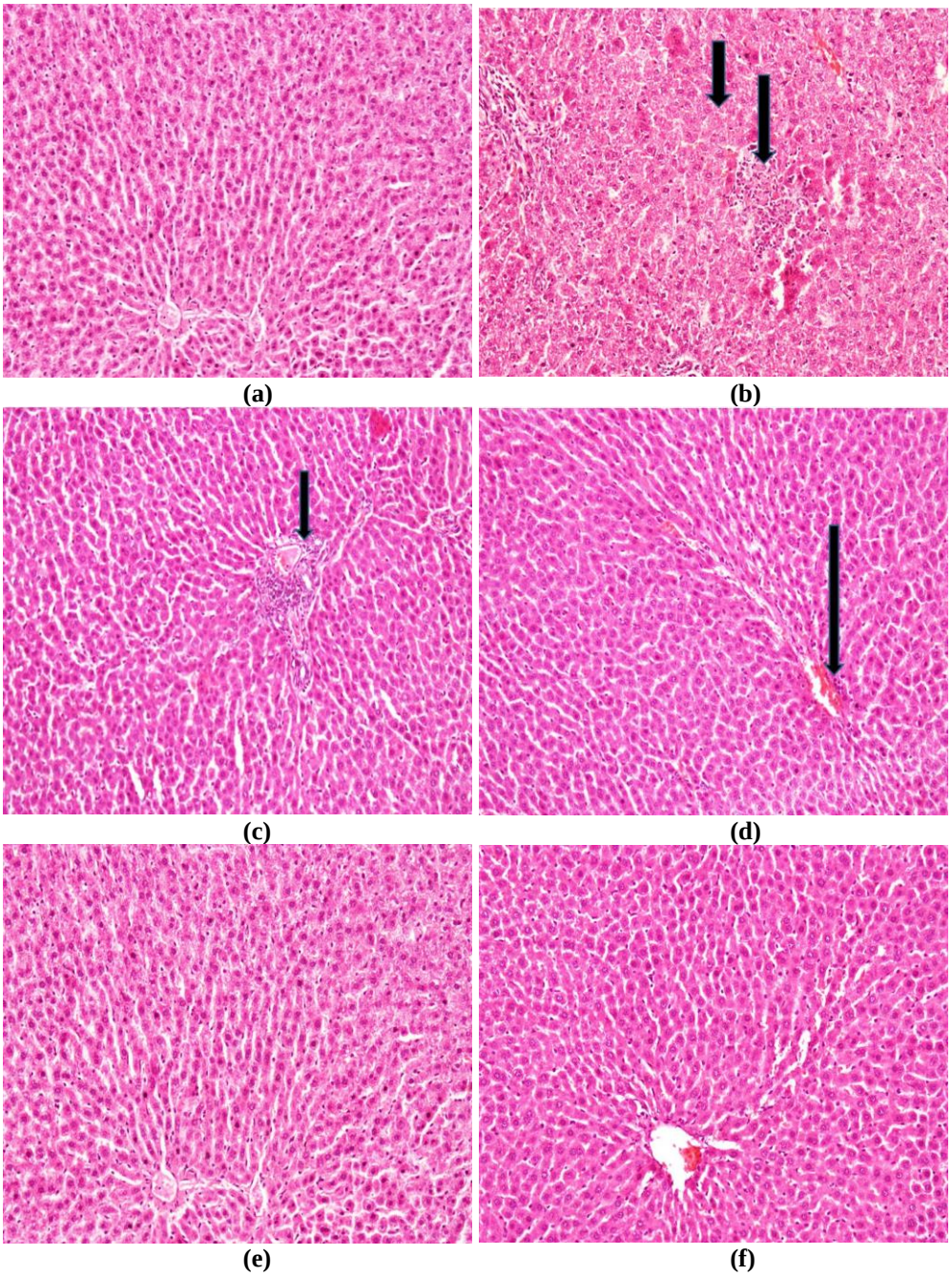
Fig 1: Histological sections of liver (14 days study): (a), (e) and (f) normal hepatocytes arranged in cord pattern in liver of naive, *Withania somnifera* and resveratrol groups respectively; (b) congestion, mild perivascular reaction, mild fatty change and sinusoidal dilation in C + D group; (c) mild vascular change and sinusoidal dilation in C + D + W group; (d) mild perivascular reaction in C + D + R group (H & E x 200)





(e) **(f)**

Fig 2: Histological section of kidney (14 days study): (a), (e) and (f) intact glomeruli and renal tubules in the cortical area of kidneys in naive, *Withania somnifera* and resveratrol groups; (b) hypercellularity in glomeruli and hyaline degenerative change in parietal layer of glomerulus with mild infilteration in interstitium in C +D group; (c) mild congestion in C+ D + W group; (d) mild congestion in glomerular capillaries in C + D + R group (H & E x 200)



(a) **(b)**

(c) **(d)**

(e) **(f)**

Fig 3: Histological sections of liver (28 days study): (a), (e) and (f) normal hepatocytes arranged in cord pattern in liver of naive, *Withania somnifera* and resveratrol groups respectively; (b) focal area of necrosis of hepatocytes with mild infiltration of MNCs in C + D group; (c) lymphocytic infilteration in perivascular area in C + D + W group; (d) mild congestion in C + D + R group (H & E x 200)

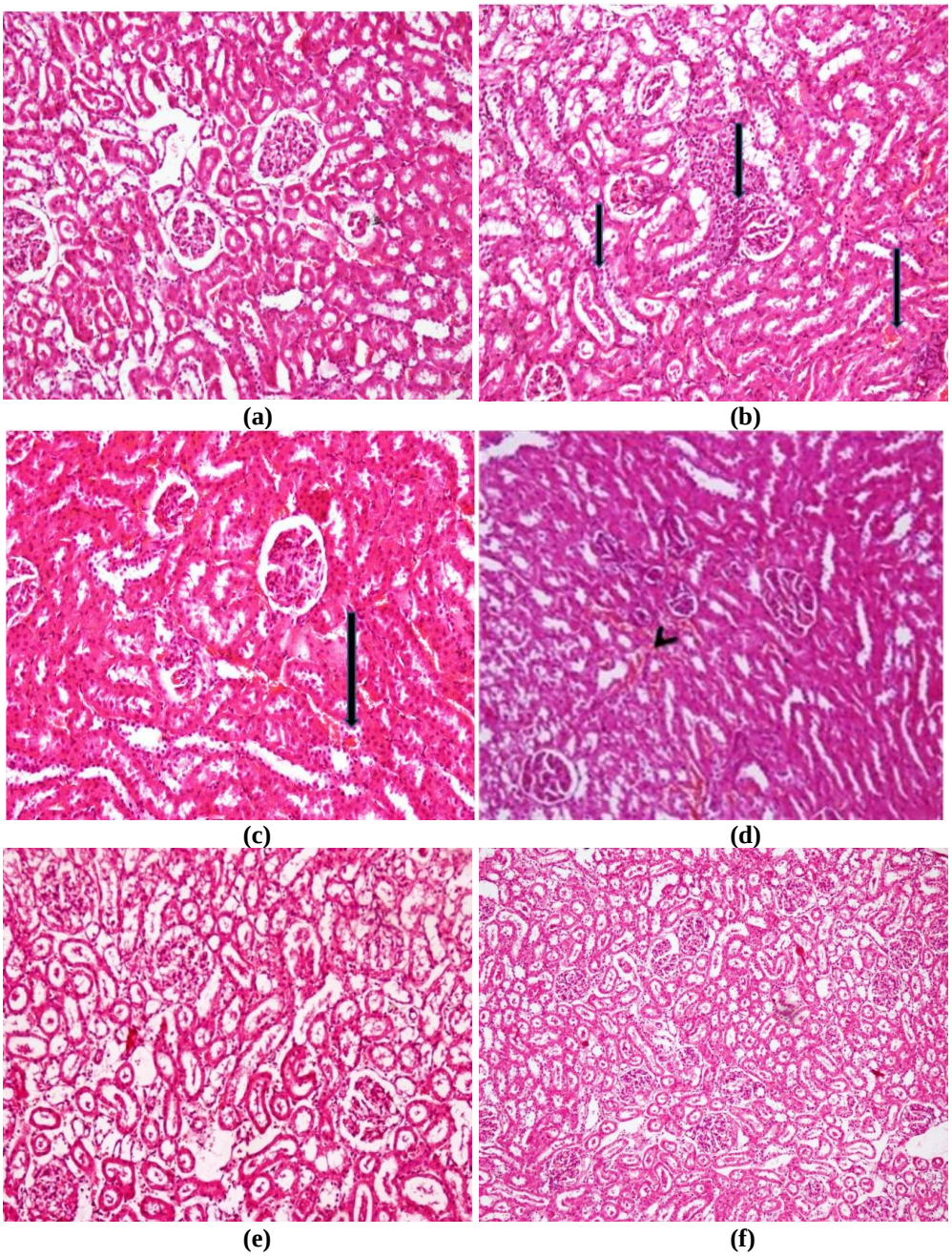


Fig 4: Histological section of kidney (28 days study): (a), (e) and (f) intact glomeruli and renal tubules in the cortical area of kidneys in naive, *Withania somnifera* and resveratrol groups; (b) focal interstitial nephritis with congestion and infiltration of mononuclear cells in C + D group; (c) mild congestion in C + D + W group; (d) showing intact glomeruli and interstitial congestion in C + D + R group (H & E x 200)

3.2.2 Kidney

Histopathological lesions in the kidney of naive and other treatment groups are presented in Figure 4. Histopathological investigations demonstrated that naive, *Withania somnifera* and resveratrol group have intact glomeruli and renal tubules in the cortical area of kidneys whereas moderate degenerative changes were seen in combined cypermethrin and deltamethrin treated group *viz*. focal interstitial nephritis and congestion. Resveratrol co-treatment and *Withania somnifera* co-treatment in combined cypermethrin and deltamethrin treatment groups showed improvement in histopathological changes of kidney by diminishing the interstitial nephritis and congestion in the kidney.

4. Discussion

Combined treatment with cypermethrin and deltamethrin seems to produce oxidative stress in liver and kidney of male rats. These findings are correlated with the histopathological changes observed in liver and kidney of combined cypermethrin and deltamethrin treated male rats as compared to naive.

4.1 Liver

Exposure of male rats to combined exposure of cypermethrin and deltamethrin produced degenerative changes in the hepatocytes characterized by congestion, telangiectiasis, mild fatty changes, focal necrosis of hepatocytes and perivascular reaction in liver. These results are in accordance with the histopathological lesions observed in the liver of broiler chicks [1], rats [10, 14] on exposure of cypermethrin and rats [11, 5] on exposure of deltamethrin.

In the present study, resveratrol and *Withania somnifera* co-treatment attenuated the histopathological changes in liver of combined cypermethrin and deltamethrin treated animals.

4.2 Kidney

Exposure of male rats to combined cypermethrin and deltamethrin produced degenerative changes in kidney such as congestion, hypercellularity in glomeruli, focal interstitial nephritis and mild reaction in kidney. These results are in accordance with the histopathological lesions observed in the kidney of rats [7, 11] and in male dwarf goats on exposure of cypermethrin [8] and in rats on exposure of deltamethrin [13].

In the present study, resveratrol and *Withania somnifera* co-treatment attenuated the histopathological changes in kidney of combined cypermethrin and deltamethrin treated rats.

5. Summary and Conclusion

Histopathological findings revealed that combined treatment of cypermethrin and deltamethrin produced a mild to moderate degenerative changes in liver and kidney which were reversed by resveratrol and *Withania somnifera*. Liver histopathological sections showed mild degenerative changes including sinusoidal dilation, congestion, fatty change and mild perivascular reaction in 14 days study while focal necrosis of hepatocytes, mild perivascular reaction were seen in 28 days study. Histopathological findings in kidney revealed hypercellularity in glomeruli and hyaline degenerative change in parietal layer of glomerulus with mild infilteration of MNCs in interstitium in 14 days study, while focal interstitial nephritis, congestion were seen in 28 days study.

In conclusion, firstly combined treatment of cypermethrin and deltamethrin significantly caused alteration in histological structure of liver and kidney as compared to naive group in both 14 days as well as in 28 days study. Secondly, resveratrol co-treatment and *Withania somnifera* co-treatment significantly ameliorated the toxic effect of combined cypermethrin and deltamethrin exposure in adult male rats in both 14 days as well as in 28 days study.

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