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Histopathology changes induced by acute and subacute toxicity of thiacloprid and its amelioration by resveratrol treatment

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Abstract

This experiment was conducted to evaluate the ameliorating effect of resveratrol against induced histopathological changes by thiacloprid in rats. Thiacloprid, a neonicotinoid insecticide is known to target the nicotinic acetyl choline receptors (nAChRs) in insects and potentially in mammals. The aim was to investigate the effect of acute (24 h) and subacute (28 days) toxicity of thiacloprid and its amelioration by resveratrol in male Wistar rats. Histopathological alterations were observed in kidney, liver, brain and spleen in both acute and subacute studies due to thiacloprid exposure which were attenuated by resveratrol co-treatment. The study revealed that thiacloprid possesses mild to moderate toxicity potential for hepatic, renal, spleen and CNS of adult male rats. Resveratrol possesses potential to sufficiently ameliorate the toxicity produced by thiacloprid and as such do not have any toxic effect at therapeutic doses in adult male wistar rats.

Keywords: Thiacloprid, resveratrol, liver, kidney, spleen and brain

1. Introduction

Neonicotinoids are a class of neuroactive insecticides and is based on their similarity to nicotine in terms of structure and action [9]. Thiacloprid [3-(6-chloro-3-pyridylmethyl)-1, 3-thiazolidin-2-ylidencyanamide] is neurotoxic and a new neonicotinoid insecticide that belongs to a new group of active ingredients, the cyanoamidines [11] and is effective on contact and also via stomach action. It produces its action by binding agonistically to the nAChRs in the CNS of insects [7, 13]. It opens the ion pores of the receptor to induce a depolarization of nerve cell membrane that triggers an action potential and abnormal excitement in the insect by interrupting the normal synaptic transmission. This mode of action is unique to the neonicotinoids, so cross-resistance to conventional insecticides is non-existent.

Resveratrol is a fat soluble compound that occurs in *trans* and *cis* configuration. Resveratrol provides protection from free-radical damage, inhibition of inflammation such as in arthritis, inhibition of the cyclooxygenase-2 (COX-2) enzyme, relaxation and protection of blood vessels, improvement of cardiovascular health [5].

2. Materials and Methods

2.1 Drugs and chemicals

Thiacloprid was purchased from Bayer Crop Science Ltd., India. Resveratrol was procured from Sigma-Aldrich Company

2.2 Animals and experimental design

Adult male Wistar rats weighing 100-120g were procured from Disease Free Small Animal House (DFSAH), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar. A total of 112 rats were used in the study. Rats were housed in polyacrylic cages in a group of 6 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 experiment was conducted. Animal house temperature varied between 22 to 27 °C throughout the study. The prior approval of institutional animal ethics committee was obtained for the use of experimental animals in this study.

2.3 Experimental design

Acute and subacute toxicity of thiacloprid and its amelioration by resveratrol was studied in adult male Wistar rats weighing 100-120g.

2.3.1 Experiment 1: Rats were divided in 6 groups for study of acute toxicity (24hrs), each comprising of 6 rats.

Group 1: Naive control: 3% gum acacia suspension was given orally in a single dose.

Group 2: Resveratrol (20mg/kg): Resveratrol (20mg/kg) in 3% gum acacia was administered orally in a single dose.

Group 3: Thiacloprid (40% of MTD): Thiacloprid suspension (40% of MTD) in 3% gum acacia was administered orally in a single dose.

Group 4: Thiacloprid (20% of MTD): Thiacloprid suspension (20% of MTD) in 3% gum acacia was administered orally in a single dose.

Group 5: Thiacloprid (40% of MTD) +Resveratrol (20mg/kg): Resveratrol (20mg/kg) and thiacloprid suspension (40% of MTD) in 3% gum acacia were administered separately in a single dose by oral route.

Group 6: Thiacloprid (20% of MTD) +Resveratrol (20mg/kg): Resveratrol (20mg/kg) and thiacloprid suspension (20% of MTD) in 3% gum acacia were administered separately in a single dose by oral route.

2.3.2 Experiment 2: Rats were divided in 6 groups for study of subacute toxicity (28days), each comprising of 6 rats.

Group 1: Naive control: 3% gum acacia suspension was given once daily orally for 28 days.

Group 2: Resveratrol (2mg/kg): Resveratrol (2mg/kg) in 3% gum acacia was administered orally in a single dose for 28 days.

Group 3: Thiacloprid (MTD/10): Thiacloprid suspension (MTD/10) in 3% gum acacia was administered once daily orally for 28 days.

Group 4: Thiacloprid (MTD/20): Thiacloprid suspension (MTD/20) in 3% gum acacia was administered once daily orally for 28 days.

Group 5: Thiacloprid (MTD/10) +Resveratrol (2mg/kg): Resveratrol (2mg/kg) and thiacloprid suspension (MTD/10) in 3% gum acacia were administered separately once daily orally for 28 days.

Group 6: Thiacloprid (MTD/20) +Resveratrol (2mg/kg): Resveratrol (2mg/kg) and thiacloprid suspension (MTD/20) in 3% gum acacia were administered separately once daily orally for 28 days.

A gap of 12 hours was maintained between resveratrol and thiacloprid administration

2.4 Sampling

Animals were sacrificed under ether anaesthesia and just after

sacrificing, vital organs *viz.* liver, kidney and brain were excised 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 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 method. The section were examined for the pathological findings of hepatic, renal, spleen and brain changes.

2.5 Statistical analysis

Data were expressed as mean \pm SE. Statistical analysis of data were performed using SPSS v16 software. Data were analyzed by analysis of variance and difference between the means was compared with Duncan's multiple comparison post hoc test. A value of $p < 0.05$ was considered statistically significant.

3. Histopathological studies

Histopathological lesions were studied in liver, kidney, brain and spleen of male rats.

3.1 In 24 h study

3.1.1 Liver: Histopathological lesions in the liver of naive and other treatment groups are presented in Fig 1. Histopathological investigations demonstrated that thiacloprid treated groups have severe to moderately diffused vacuolar degeneration of hepatocytes with pyknotic nuclei in liver. Resveratrol co-treatment in thiacloprid treatment groups provided improvement in histopathological changes of liver by diminishing vacuolar degeneration of hepatocytes.

3.1.2 Kidney: Histopathological lesions in the kidney of naive and other treatment groups are presented in Fig 2. Histopathological investigations demonstrated that naive and RT group have intact glomeruli and renal tubules in the cortical area of kidneys whereas thiacloprid treated groups have focal loss of tubules due to degeneration and haemorrhages in the cortical area of kidney. Resveratrol co-treatment in thiacloprid treatment groups provided improvement in histopathological changes of kidney by diminishing the degeneration and haemorrhages in the cortical area of kidney.

3.1.3 Brain: Histopathological lesions in the brain of naive and other treatment groups are presented in Fig 3. Histopathological investigations demonstrated that naive and RT group have intact neuron and glial cells in the neuropil of cerebral cortex whereas thiacloprid treated groups have neuronal degeneration and darkly stained neurons in the neuropil of cerebral cortex. Resveratrol co-treatment in thiacloprid treatment groups provided improvement in histopathological changes of brain by diminishing the neuronal degeneration and darkly stained neurons in the neuropil of cerebral cortex.

3.1.4 Spleen: Histopathological lesions in the spleen of naive and other treatment groups are presented in Fig.4. Histopathological investigations demonstrated that naive and RT group have normal red pulp and adequate lymphocytes in PALS of white pulp in the spleen whereas thiacloprid treated groups have mild lymphocytic depletion in PALS of white pulp in the spleen. Resveratrol co-treatment in thiacloprid

treatment groups provided improvement in histopathological changes of spleen by diminishing the lymphocytic depletion in PALS of white pulp.

3.2 In 28 days study

3.2.1 Liver: Histopathological lesions in the liver of naive and other treatment groups are presented in Fig.5. Histopathological investigations demonstrated that naive and RT group have normal hepatocytes arranged in cord pattern around the central vein whereas thiacloprid treated groups have severely diffused vacuolar degeneration of hepatocytes with pyknotic nuclei in liver. Resveratrol co-treatment in thiacloprid treatment groups provided improvement in histopathological changes of liver by diminishing vacuolar degeneration of hepatocytes.

3.2.2 Kidney: Histopathological lesions in the kidney of naive and other treatment groups are presented in Fig.6. Histopathological investigations demonstrated that naive and RT group have intact glomeruli and renal tubules in the cortical area of kidneys whereas thiacloprid treated groups have degeneration and formation of hyaline cast in the lumen of tubules in cortical area of kidney. Resveratrol co-treatment in thiacloprid treatment groups provided improvement in histopathological changes of kidney by diminishing the degeneration and haemorrhages in the cortical area of kidney.

3.2.3 Brain: Histopathological lesions in the brain of naive and other treatment groups are presented in Fig.7. Histopathological investigations demonstrated that naive and RT group have intact neuron and glial cells in the neuropil of cerebral cortex whereas thiacloprid treated groups have marked neuronal degeneration and congestion of blood vessels in the neuropil of cerebral cortex. Resveratrol co-treatment in thiacloprid treatment groups provided improvement in histopathological changes of brain by diminishing the neuronal degeneration in the neuropil of cerebral cortex.

3.2.4 Spleen: Histopathological lesions in the spleen of naive and other treatment groups are presented in Fig.8. Histopathological investigations demonstrated that naive and RT group have normal red pulp and adequate lymphocytes in PALS of white pulp in the spleen whereas thiacloprid treated groups have moderate to mild lymphocytic depletion in PALS of white pulp in the spleen. Resveratrol co-treatment in thiacloprid treatment groups provided improvement in histopathological changes of spleen by diminishing the lymphocytic depletion in PALS of white pulp.

4 Discussion

Effect of acute and subacute toxicity of thiacloprid on histopathological changes in organs and its amelioration by resveratrol in male rats

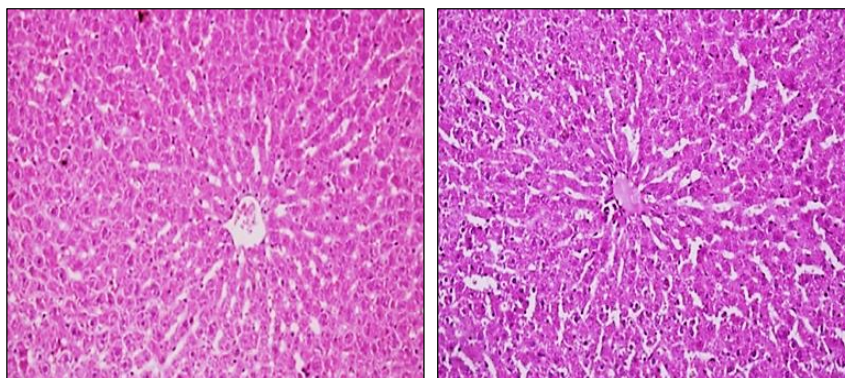
Thiacloprid seems to produce oxidative stress in liver, kidney, brain and spleen of male rats. These findings also seem to be correlated with the histopathological changes observed in various organs of male rats as compared to naive animals.

4.1 Liver: Exposure of male rats to thiacloprid produced degenerative changes in the hepatocytes characterized by vacuolar degeneration and pyknotic nuclei in liver. These results are also in accordance with the histopathological lesions observed in the liver of male rats [4]. Goyal *et al.* (2010) [3] reported significant histopathological alterations in liver, such as mild fatty changes, congestion and degeneration of hepatocytes after thiacloprid toxicity in *Gallus Domesticus*. Similar histopathological changes were observed after 90 days oral exposure of imidacloprid in female rats [1] and after 4 weeks oral administration of imidacloprid in male albino rats [8, 10]. In the present study, resveratrol co-treatment attenuated the histopathological changes in liver of imidacloprid treated animals. Our results are in agreement with the observations of Grissa *et al.* (2007) [2] where resveratrol attenuated oxidative stress and histological alterations induced by liver ischemia/reperfusion in rats.

4.2 Kidney: Exposure of male rats to thiacloprid produced degenerative changes in kidney such as degeneration and formation of hyaline cast in the lumen of tubules, congestion and haemorrhages in cortical area of kidney. Goyal *et al.* (2010) [3] reported alterations in histoarchitecture of kidney which included marked congestion, tubular cell degeneration and sloughing of epithelial cells in *Gallus Domesticus* due to thiacloprid toxicity.

4.3 Brain: Acute and subacute toxicity of thiacloprid resulted in neuronal degeneration and congestion of blood vessels in the neuropil of cerebral cortex. Similar findings were reported by Goyal *et al.* (2010) [3] where cerebral hemisphere revealed changes comprising of mild neuronal degeneration with surrounding glial cells in *Gallus Domesticus* due to thiacloprid toxicity. Similar findings were reported by Soujanya *et al.* (2012) [10] where imidacloprid treatment resulted in histological and ultrastructural alterations in brain of male rats.

Resveratrol co-treatment restored the normal histopathological structure of brain. It could be due to inhibition of delayed neurological damaged and decreased glial cell activation by resveratrol [12]. These results demonstrate resveratrol having neuro-protective action.



a

b

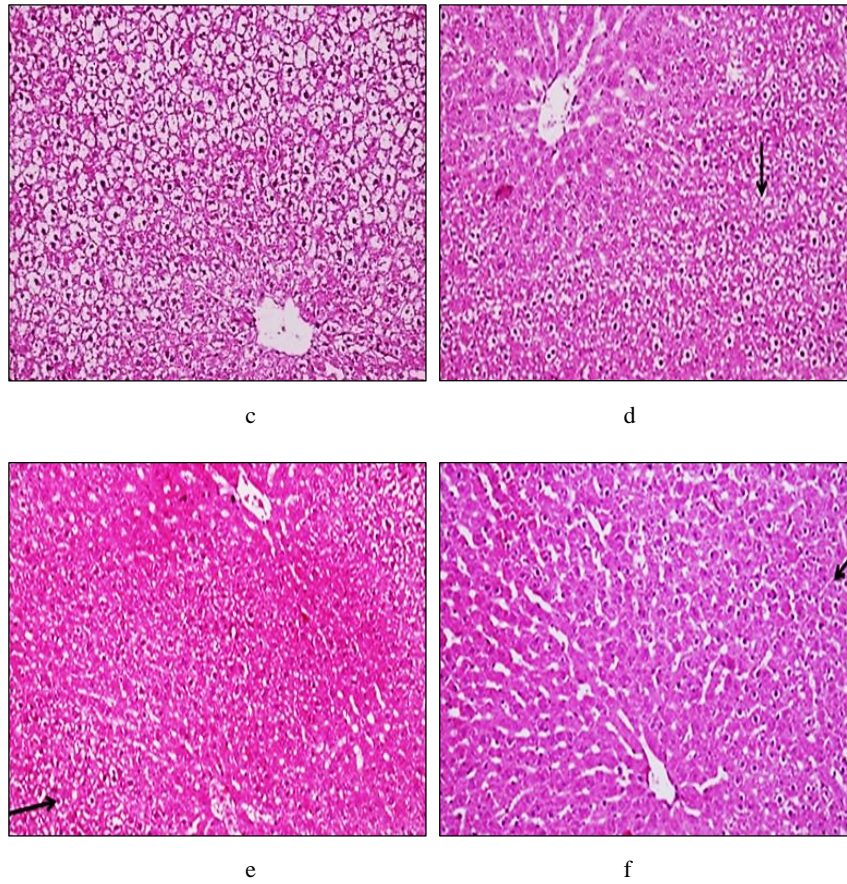
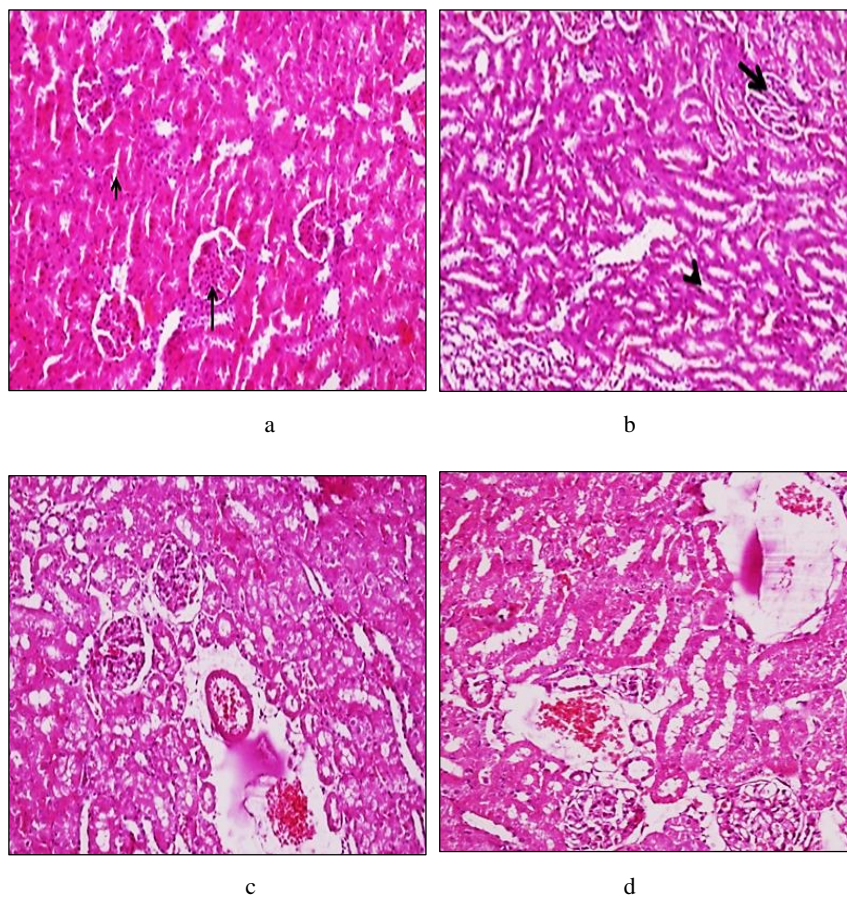


Fig 1: Histological sections of liver (24 h exposure): (a) and (b) normal hepatocytes arranged in cord pattern around the central vein in liver of naïve and RT groups respectively; (c) severe diffuse vacuolar degeneration of hepatocytes with pyknotic nuclei in 40% THIA group; (d) moderate diffuse vacuolar in degeneration of hepatocytes in midzonal region (arrow) of hepatic lobule of liver in 20% THIA group; (e) mild diffuse vacuolar degeneration of hepatocytes in midzonal region (arrow) of hepatic lobule of liver in 40% THIA + RT group; (f) mild degeneration of hepatocytes in midzonal region (arrow) of hepatic lobule of liver in 20% THIA + RT group (H & E x 200)



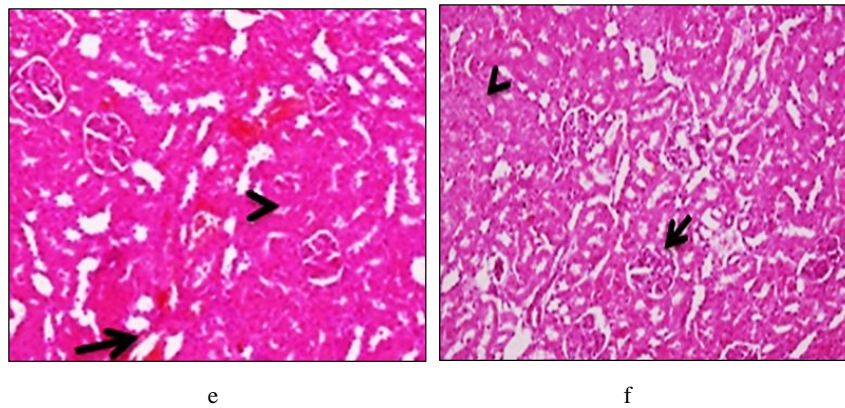


Fig 2: Histological sections of kidney (24 h exposure): (a) and (b) intact glomeruli (arrow) and renal tubules (arrow head) in the cortical area of kidneys in naïve and RT groups respectively; (c) congestion and haemorrhages in kidney in 40% THIA group; (d) focal loss of tubules due to degeneration and haemorrhage in the cortical area of kidney in 20% THIA group; (e) focal area of haemorrhage (arrow) and swelling of tubular epithelium (arrow head) in the cortical area of kidneys in 40% THIA + RT group; (f) intact glomeruli (arrow) and mild swelling of renal tubules (arrow head) in the cortical area of kidney 20% THIA + RT group (H & E x 200)

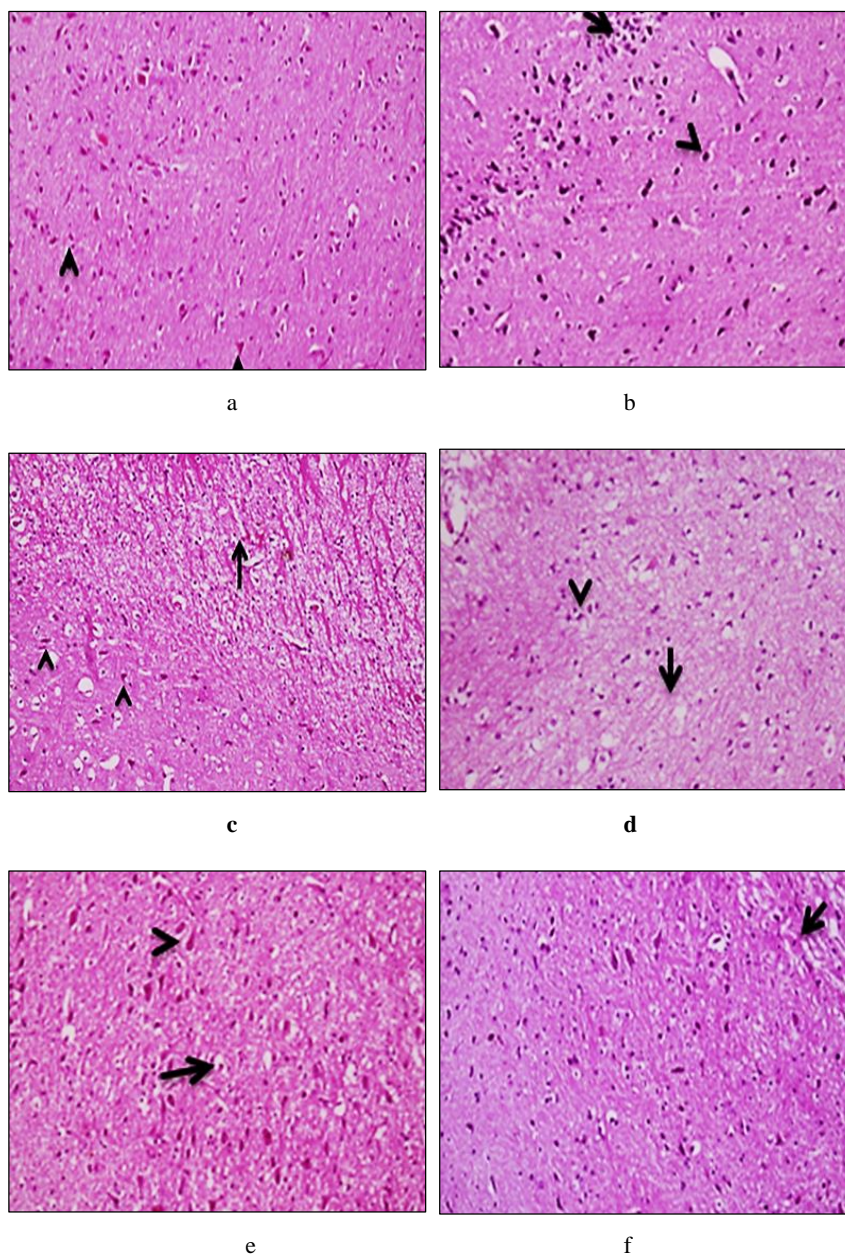


Fig 3: Histological sections of brain (24 h exposure): (a) and (b) intact neurons (arrow) and glial cells (arrow head) in the neuropil of cerebral cortex in naïve and RT groups respectively; (c) marked neuronal degeneration (arrow) and darkly stained neurons (arrow head) in the neuropil of cerebral cortex in 40% THIA group; (d) moderate neuronal degeneration (arrow) and darkly stained neurons (arrow head) in the neuropil of cerebral cortex in 20% THIA group; (e) moderate neuronal degeneration (arrow) and darkly stained neurons (arrow head) in the neuropil of cerebral cortex 40% THIA + RT group; (f) mild neuronal degeneration (arrow) in the neuropil of cerebral cortex in 20% THIA + RT group (H & E x 200)

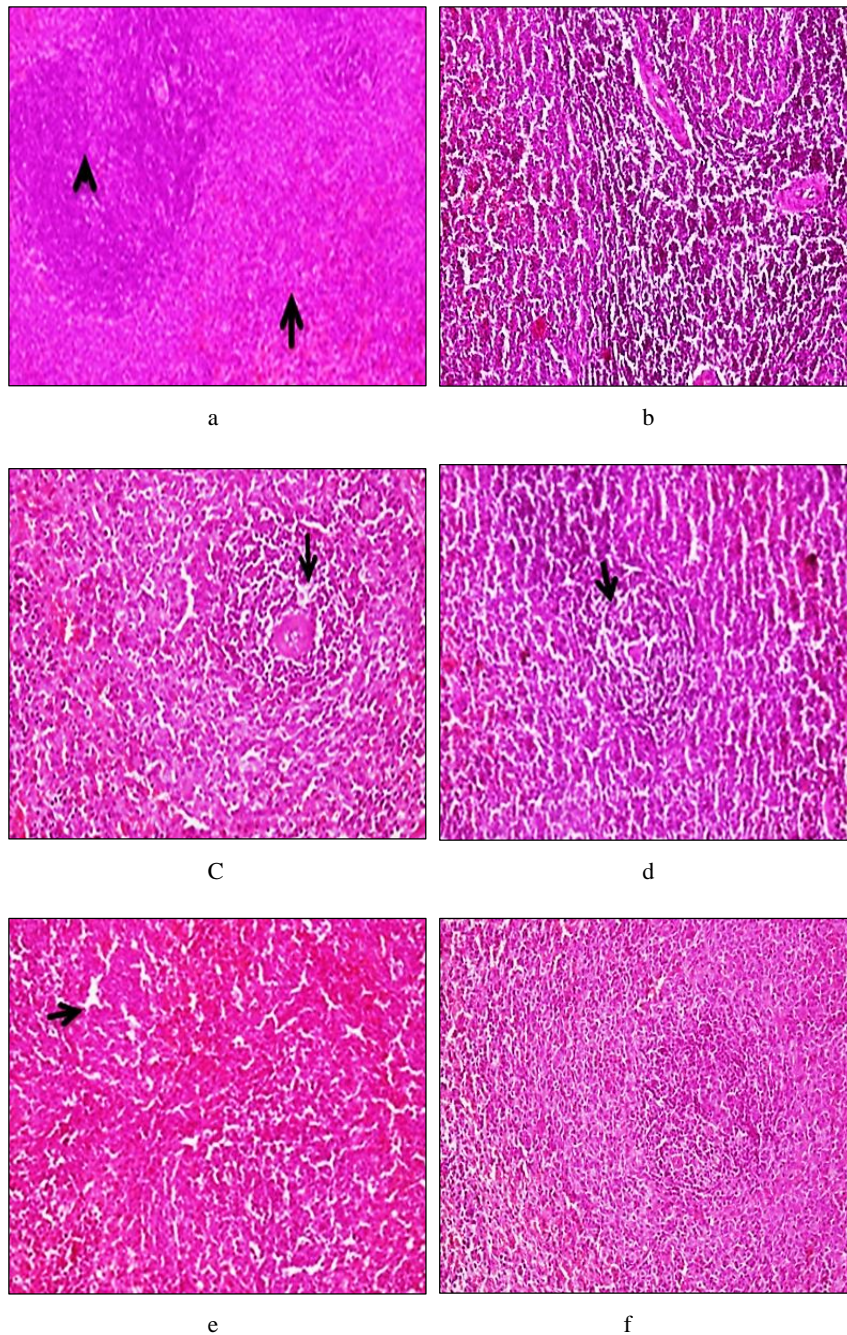
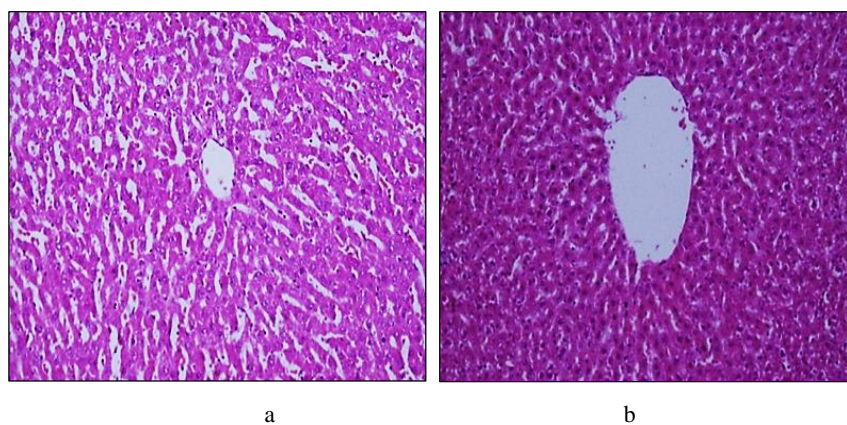


Fig 4: Histological sections of spleen (24 h exposure): (a) normal red pulp (arrow) and adequate lymphocytes in PALS of white pulp (arrow head) in the spleen in naïve group (b) showing mild deletion of lymphocytes in PALS of white pulp in the spleen in RT group; (c) mild lymphocytic depletion in PALS of white pulp (arrow) in the spleen in 40% THIA group; (d) mild lymphocytic depletion in PALS of white pulp (arrow) in the spleen in 20% THIA group; (e) very mild depletion of lymphocytes in PALS of white pulp in the spleen in 40% THIA + RT group; (f) adequate lymphocytes in PALS of white pulp in the spleen in 20% THIA + RT group (H & E x 200)



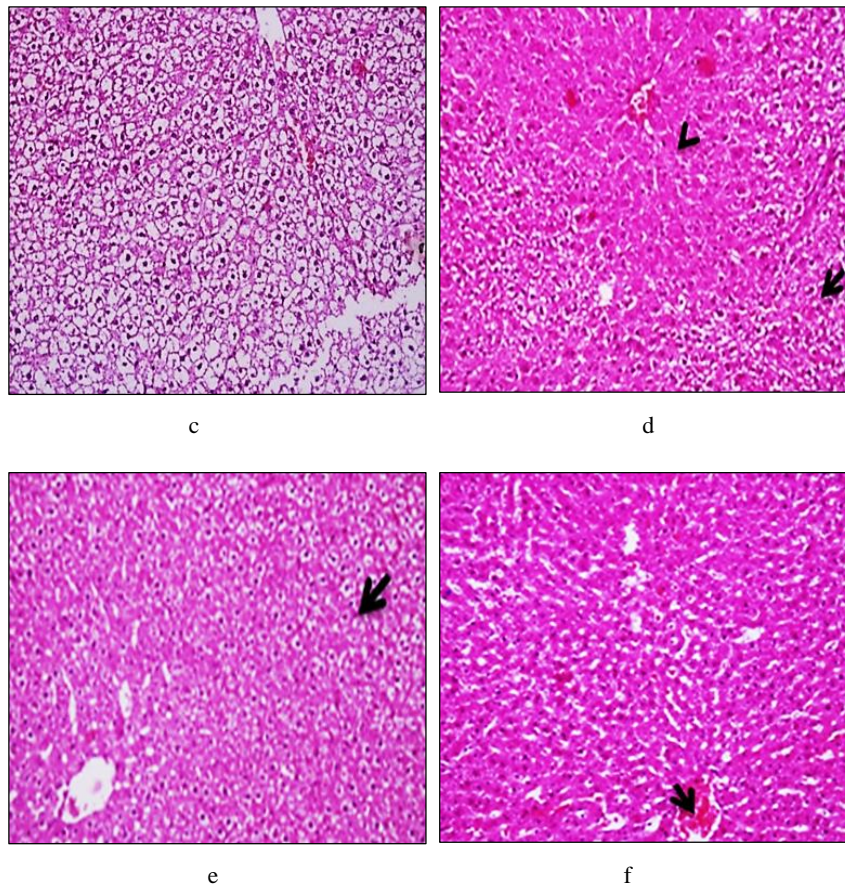
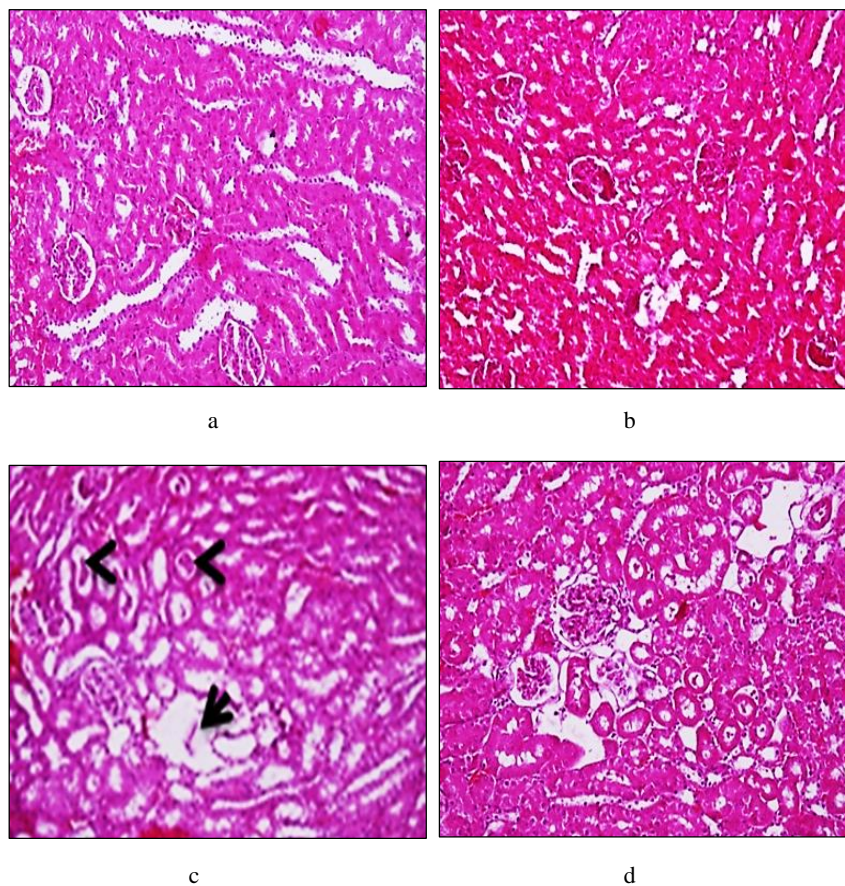


Fig 5: Histological sections of liver (28 days study): **(a) and (b)** normal hepatocytes arranged in cord pattern around the central vein in liver in naïve group and RT respectively; **(c)** severe diffuse vacuolar degeneration of hepatocytes in liver 10% THIA group; **(d)** moderate diffuse vacuolar degeneration of hepatocytes in midzonal region (arrow) and swelling of hepatocytes in centrilobular region (arrow head) of liver in 5% THIA group; **(e)** mild vacuolar degeneration of hepatocytes in midzonal region (arrow) of liver in 10% THIA + RT group; **(f)** congestion of central vein (arrow) and hepatocytes arranged in cord pattern in liver in 5% THIA + RT group (H & E x200)



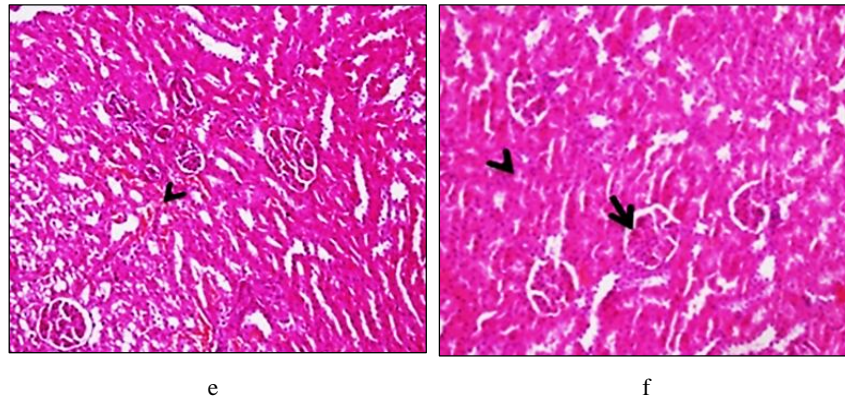


Fig 6: Histological sections of kidney (28 days study): (a) and (b) intact glomeruli and renal tubules in the cortical area of kidney naïve and RT groups; (c) showing degeneration (arrow) and formation of hyaline cast (arrow head) in the lumen of tubules in cortical area of kidney in 10% THIA group; (d) degeneration and swelling of tubules in the cortical area of kidney in 5% THIA group; (e) showing intact glomeruli and focal haemorrhage (arrow head) in the cortical area of kidney in 10% THIA + RT group; (f) glomeruli (arrow) and mild swelling of renal tubules (arrow head) in the cortical area of kidney in 5% THIA + RT group (H & E x 200)

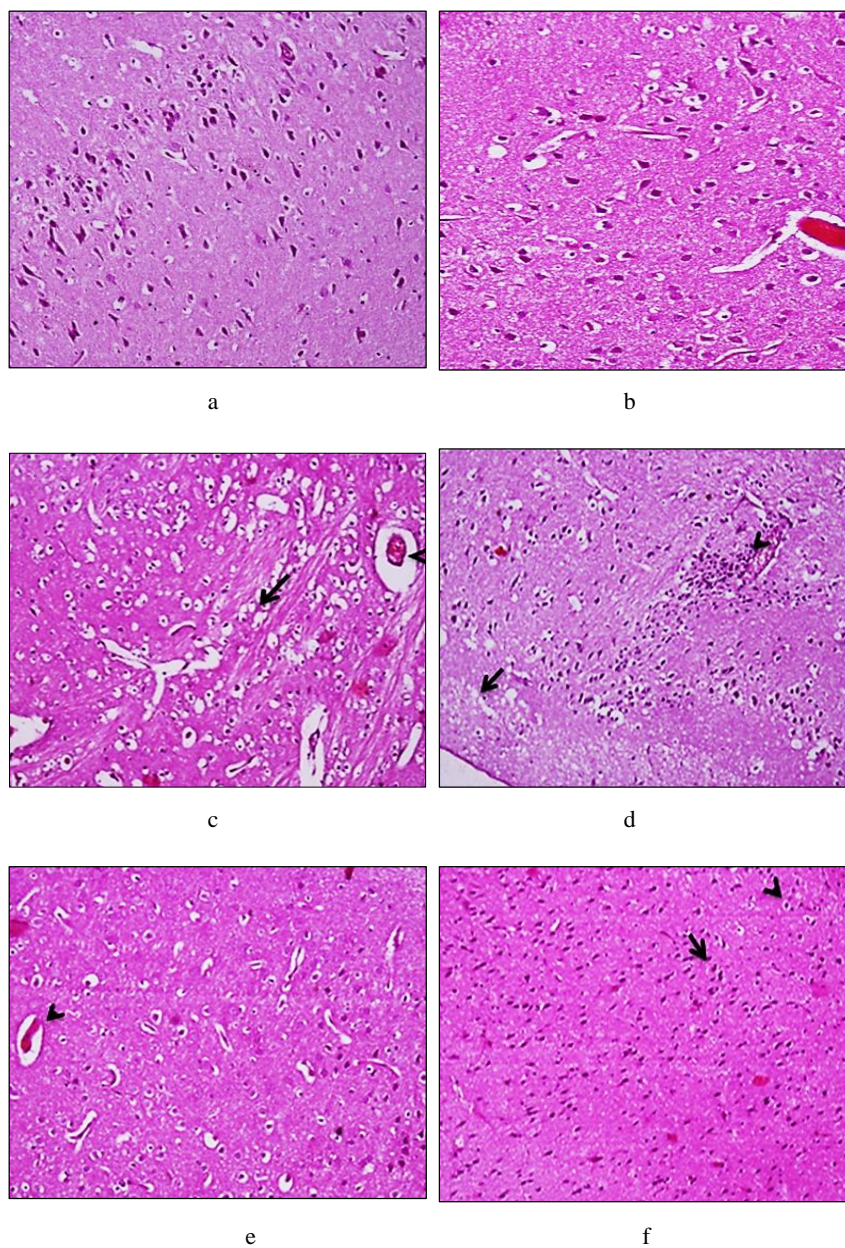


Fig 7: Histological sections of brain (28 days study): (a) and (b) intact neurons and glial cells in the neuropil of cerebral cortex in naïve and RT groups respectively; (c) marked neuronal degeneration (arrow) and congestion of blood vessels (arrow head) in the neuropil of cerebral cortex in 10% THIA group; (d) moderate neuronal degeneration (arrow) and congestion of blood vessels (arrow head) in the neuropil of cerebral cortex in 5% THIA group; (e) congestion of blood vessel (arrow head) in the neuropil of cerebral cortex in 10% THIA + RT group; (f) intact neurons (arrow) and glial cells (arrow head) in the neuropil of cerebral cortex in 5% THIA + RT group (H & E x 200)

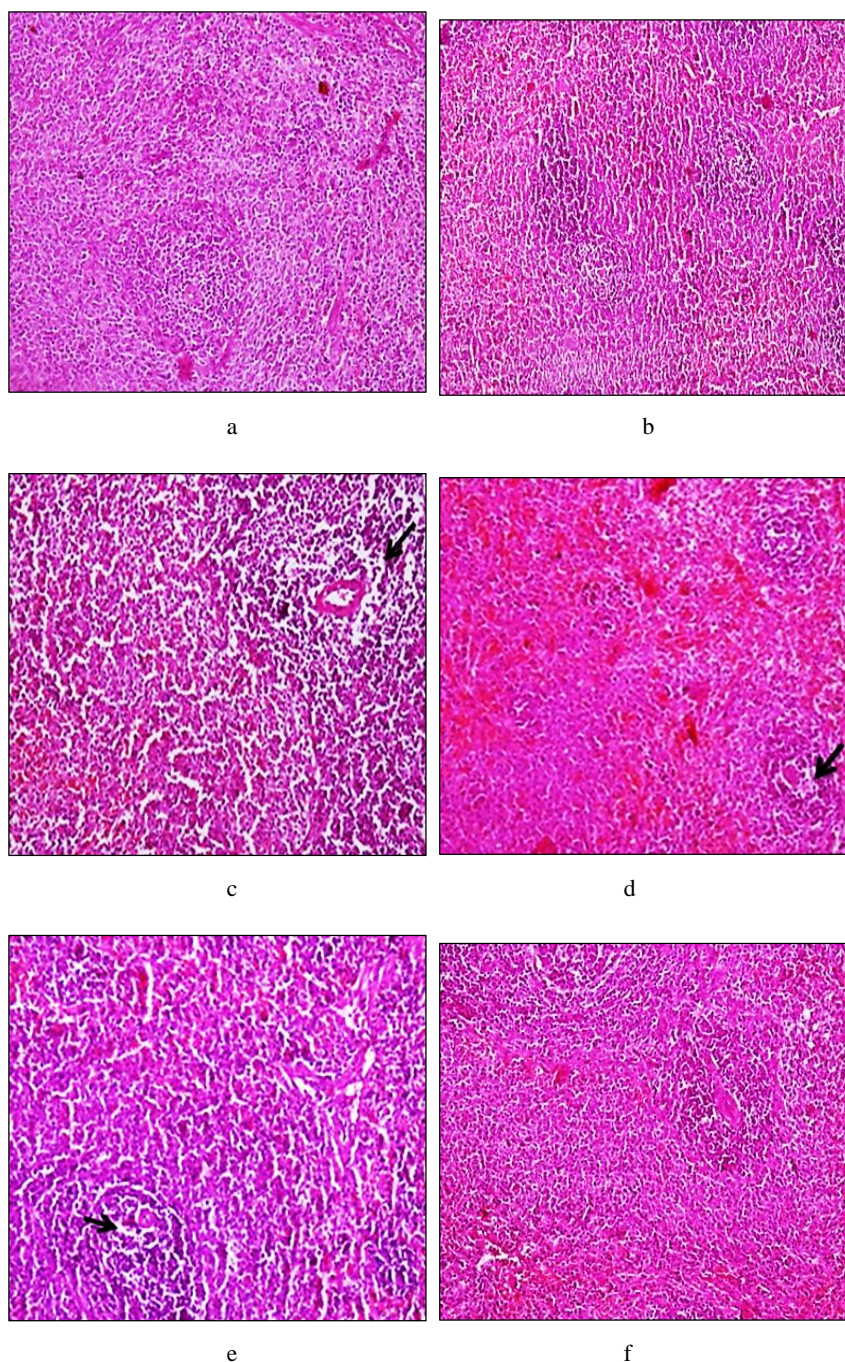


Fig 8: Histological sections of spleen (28 days study): (a) and (b) normal red pulp and adequate lymphocytes in PALS of white pulp in the spleen in naïve and RT groups respectively; (c) showing moderate depletion of lymphocytes (arrow) in PALS of white pulp in the spleen in 10% THIA group; (d) mild depletion of lymphocyte in germinal centre (arrow) of PALS of white pulp in the spleen in 5% THIA group; (e) mild depletion of lymphocytes in PALS of white pulp in the spleen in 10% THIA + RT group; (f) adequate lymphocytes in PALS of white pulp in the spleen in 5% THIA + RT group (H & E x 200)

4.4 Spleen: Acute and subacute toxicity of thiacloprid resulted in moderate to mild lymphocytic depletion in PALS of white pulp in the spleen. Our findings are in agreement with Mohany *et al.* (2011) [8] who reported that histopathologically, the spleen tissues of the imidacloprid-treated rats displayed low numbers of lymphocytes, some of which appeared to be pyknotic.

Resveratrol co-treatment in thiacloprid treated groups restored the normal histopathological structure of spleen. It may be attributed to the protective effect of resveratrol on spleen by reducing oxidative stress [6].

5. Conclusion

Histopathological findings revealed that thiacloprid produced

a mild to moderate degenerative changes in liver, kidney, brain and spleen which were reversed by resveratrol. Liver histopathological sections showed degenerative changes including vacuolation, congestion and pyknotic nuclei. Histopathological findings revealed focal loss of tubules due to degeneration and haemorrhages in the cortical area of kidney. Histopathological examination showed neuronal degeneration and darkly stained neurons in the neuropil of cerebral cortex in brain and mild lymphocytic depletion in PALS of white pulp in the spleen. In conclusion, it is suggested that thiacloprid possesses mild to moderate toxicity potential for liver, kidney, brain and spleen in male Wistar rats. Resveratrol possesses potential to sufficiently ameliorate the toxicity produced by thiacloprid and as such do not have

any toxic effect at therapeutic doses in male Wistar rats. The exact mechanism of action of acute and subacute toxicity of thiacloprid is not fully understood as yet but it can be hypothesized that it produces multiorgan toxicity by enhancing oxidative stress, endocrinal disruption, recruitment of neutrophils and lymphocytes, lipid peroxidation,, decreasing antioxidant status and damaging histological structures along with change in hematological profile.

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