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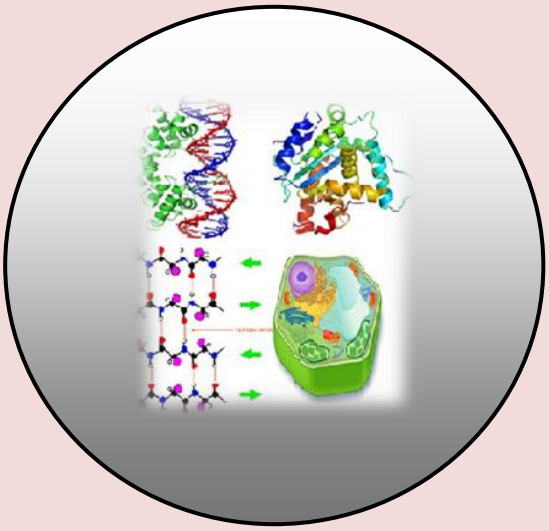

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RESEARCH PAPER

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Histopathological Changes in Swiss Albino Mice due to Dimethoate and Ameliorative Effect of Leaf Extract *Ziziphus nummularia*

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ABSTRACT

Dimethoate one of the potent organophosphorus pesticides, widely used in agriculture and households. Dimethoate effect vital organs as liver, kidney, brain, heart .The present study was undertaken to investigate the toxic effect of the Dimethoate and ameliorative properties of ZZLN leaf extract on liver and kidney of swiss albino mice. Swiss albino mice were divided in five groups as G1A group represent 70 days age groups. First group control, Group 2 dimethoate treated (30 mg/kgbw). 3. Dimethoate 30 mg/kgbw +leaf extract of ZZLN 250 ml/kgbw, 4. Dimethoate 15 mg/kg bw. 5. Dimethoate 15 mg/kg + 250 ml/kg bw. Dimethoate treated groups shows histopathological changes in the liver and kidney. There were congestion blood vessels, hemorrhages, infiltration, vasodilation, fatty change, hypertrophy in liver kidney showed some changes including Glomerular Degeneration, Tubular Degeneration, Hemorrhage, Infiltration, Hydropic Changes, Tubular Cast, Tubular Widened Lumen, Glomerular Shrinkage and Compressed Blood Vessel. The ZZLN treated groups show healing and antioxidant properties .In G3A and G5A hepatocytes in nomal architecture, no necrosis, less vasodilation, in kidney normal glomerulus, DCT, PCT with normal widening Dimethoate treated group shows the ALT, AST, ACP, ALP, Cholesterol, bilirubin value was significantly increased ($p<0.05$) . There was difference in ZZLN treated groups shows decrease in ALT, AST, ALP, ACP, Cholesterol, bilirubin value.

Key words: DCT, PCT, Dimethoate, Liver and Kidney.

INTRODUCTION

Pesticides are substances that are used to control weeds and pest. Pesticides are used to increase the production of food by eliminating the unwanted Pest and different disease vectors (Prakasam et al, 2001) Organophosphorus pesticides used extensively worldwide to control pests. Dimethoate is one of the organophosphorus pesticide used to spray on crops, vegetables, fruits and to kill house fly Tse tse fly etc.Extensive use and toxic effect of dimethoate destroy the health of soil, human and environment.

Majority of population is exposed to lower doses of dimethoate via food, contaminated drinking water, or by application of household insecticides containing dimethoate (Sharma *et al*., 2005). Organophosphorus insecticides toxicity always evaluated by change in biochemical parameters and histopathological changes in tissue and organs *(Ghanem et al., 2006; Massoud et al., 2010).* Dimethoate, (IUPAC name O, O-dimethyl SN-methyl carbamoyl methyl phosphorodithioate) is a organophosphorus pesticides widely used against a broad range of insects and mites and is also used for indoor control of houseflies spray on vegetables and fruits. The extensive use of Dimethoate poses a health hazard to animals and humans because of its persistence in soil and crops (WHO). When humans are exposed to Dimethoate, there are many effects, when inhaled, the first effects are usually respiratory and may include a bloody or runny nose, coughing, chest discomfort, difficult or short breath, and wheezing due to constriction or excess fluid in the bronchial tubes. Skin contact may cause skin sensitization. Eye contact will cause pain, bleeding, tears, pupil constriction and blurred vision. Dimethoate toxicity directly affect liver and kidney. Numerous studies indicate that dimethoate intoxication can cause oxidative stress by the generation of free radicals and induce hepatic lipid peroxidation in mice (Sivapiriya et al., 2006). The liver is at great risk of injury, as it is involved in the transformation of environmental xeno- biotics which induces hepatotoxicity. Oral administration of dimethoate to rats induced a significant hepatic damage, as observed from the alteration of hepatospecific enzyme activities. Altered cell membrane permeability of liver cells can lead to enhanced enzyme activity in plasma. *Ziziphus nummularia* belong to family Rhamnaceae. Phytochemical analysis of *Ziziphus nummularia* leaves and fruits confirm the presence of *Ziziphus nummularia* is reported to possoess antitumor (*Kumar S, et.al 2011*), anthelmintic (Bachaya, H.A, et.al 2009), antibacterial analgesic and anti-inflammatory [De Boer, HJ. et. al. 2005] properties. In the present study we investigate the histopathological change in the liver and Kidney of swiss albino mice, when exposed to Dimethoate and study the healing properties of ZZLN leaf extract.

MATERIAL AND METHOD

Healthy albino mice of age group (70 days age 52.65+_2.41) were taken for experimental study. They were acclimatized to laboratory condition (room temp 25 +_5C RH 50+_10%) for two week before starting the experiment. Each rat housed separately in a polypropylene cage (4x27x13) and offered pellated food and water ad libitum. Swiss albino mice were divided in 5 groups as G1A group represent 70 days age groups.

1. G1A Dimethoate (control).
2. G2A Dimethoate 30mg /kgbw day^{-1}
3. G3A Dimethoate 30mg/kgbw + 250 ml/kgbw day^{-1} *ziziphus nummularia* extract. The leaves extract given after 60 min of dimethoate {Rajshekharan et al. 2013}.
4. G4A Dimethoate 15mg/kgbw day^{-1}
5. G5A. Dimethoate 15mg/kgbw + 250 ml/kgbw day^{-1} *ziziphus nummularia* extract given after 60 min of dimethoate.

The experiment has continued for 30 days. At the end of experiment period blood was collected for biochemical analysis and kidney and liver removed for histological analysis.

Histopathological studies

After the experiment all mice were sacrificed. The liver and kidney were removed, washed in normal saline, fixed in bouin s fluid as a histological fixative for 24 hours. The liver washed in ethyl alcohol 70%, while the kidney washed in tap water. According to Humason (1967) the tissues were processed as usual in the recognized method of dehydration in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax. Paraffin sections were cut at 6µm thicknesses using a rotary microtome. The sections were stained with harris haematoxylin and eosin. Finally prepared sections were examined using a light microscope and photographs were taken with an automatic photo micrographic system.

Observations

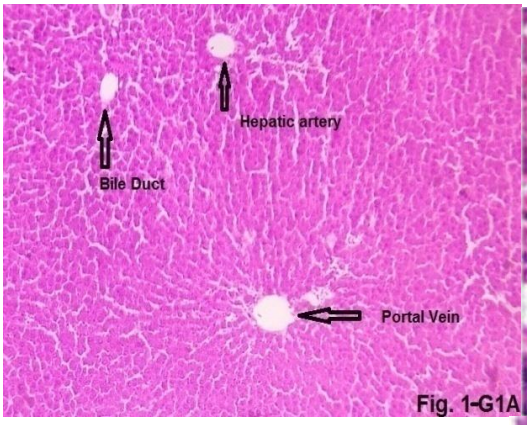


Fig. 1-G1A

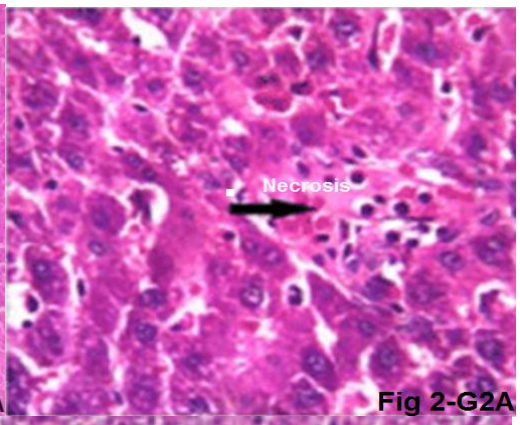


Fig 2-G2A

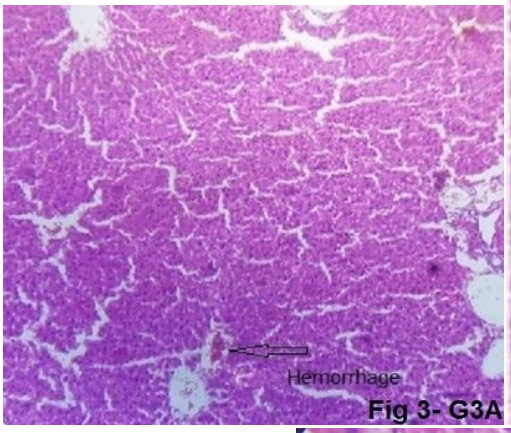


Fig 3- G3A

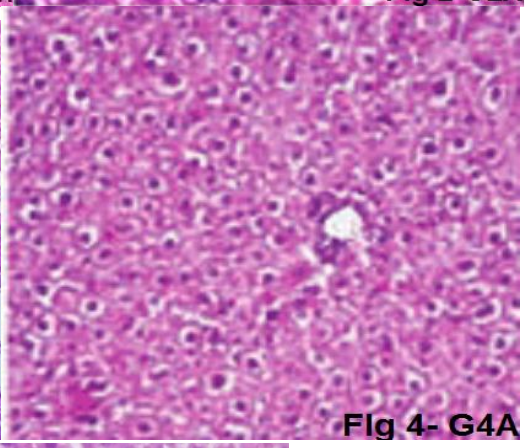


Fig 4- G4A

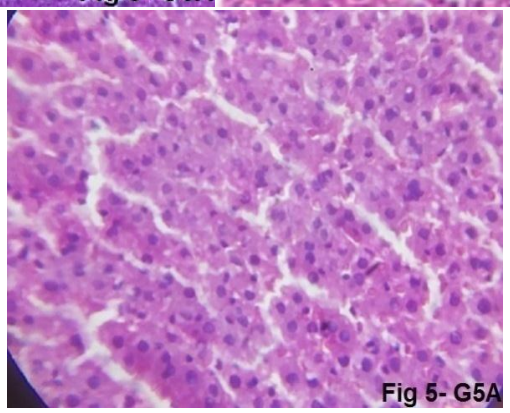


Fig 5- G5A

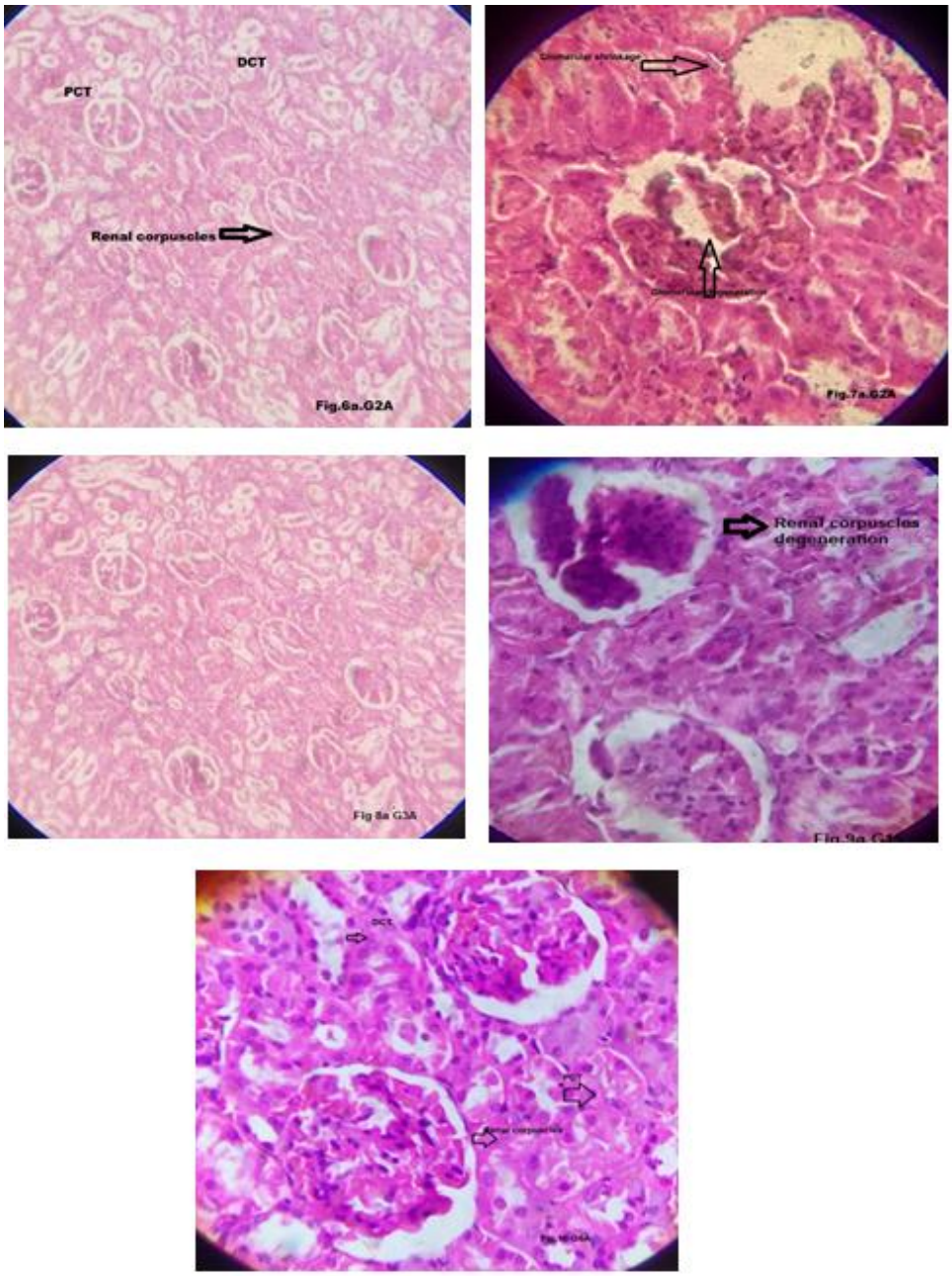
Photomicrograph of the liver sections of mice treated with Dimethoate and ZZLN H&E Stain X 650)

Figure 1. Control liver showing normal hepatocytes architecture with normal Central vein, hepatic cells sinusoids and Kupffer cells.
Figure 2. 15 mg/Kg bw of Dimethoate treated liver showed congestion and infiltration.
Figure 3. 15 mg/Kg bw of Dimethoate+ ZZLN liver showing normal hepatocytes architecture, sinusoids.
Figure 4. 30 mg/Kg bw of Dimethoate treated liver showing congestion and infiltration vasodilation, necrosis.
Figure 5. 30 mg/Kg bw of Dimethoate ,ZZLN treated liver showing normal hepatocytes architecture with normal Central vein, hepatic cells sinusoids, little vasodilation.

RESULTS AND DISCUSSION

Histopathological changes in liver

The histopathological examination results in this study demonstrated that 30-days the oral administration of Dimethoate 15mg/kgbw (1/10 of LD $_{50}$) caused many degenerative changes in the liver including congestion blood vessels, infiltration, vasodilatation and hydropic changes, Also liver section of Mice in this group treated with high dose (1/5 the. LD $_{50}$) of dimethoate showed congestion blood vessels, infiltration, hemorrhage, vasodilatation, fat deposition .The histopathological examination of the liver in the control group showed normal histological slides The liver lobule clearly seen and the central vein lies at the centre of the lobule. Lobule surrounded by hepatocytes. Hepatocytes in proper orientation with distinct nuclei and granulated cytoplasm as shown in G1A (fig 1).



Photomicrograph Of The Kidney Sections Of Mice Treated With Dimethoate ZZLN H&E Stain X 650).

Figure 6. Control Kidney Showing Normal Glomerulus and Normal Kidney Tubules
Figure 7. 15 mg/Kg bw of Dimethoate treated showing Glomerular Degeneration, Tubular Degeneration, Shrinkage of glomerulus, Hemorrhage.
Figure 8. 15 mg/Kg bw of Dimethoate + ZZLN, showing normal glomerulus, tubules, PCT, (proximal convulted tubules), DCT (distal convulted tubules).
Figure 9. 30 mg/Kg bw of Dimethoate treated group showing Glomerular Shrinkage, Infiltration, Tubular Widened Lumen And Tubular Degeneration.
Figure 10. 30 mg/Kg bw of Dimethoate + ZZLN, showing normal renal corpuscles, PCT, DCT.

Histopathological change in dimethoate treated group revealed a impairment in hepatocytes, pyknotic nuclei, fibrosis, dilated bile duct, inflammatory infiltration. The liver of Mice treated with (1/10 the LD 50) of dimethoate showed that there were liver congestion and infiltration vasodilatation and congestion and hydropic changes in G2A (Fig. 2). The ameliorative effects of ZZLN leaf extract in liver show the healing properties. Hepatocytes in well architecture, sinusoids dilated in G3A groups (fig 3a). The liver of mice treated with the 30mg/kg bw(1/5 of LD 50)Showed that architecture of hepatocytes deform, pycnosis, dilation of sinusoids, dilated bile duct ,inflammatory infiltration, hemorrhage, fat deposition in G4A (fig.4). The curing and antioxidant properties of ZZLN leaf extract in G5B groups showed in (fig 5) very less vasodilation, distinct nuclei, no hemorrhage.
The liver of mice of control group showed that the bile duct, hepatic artery, portal vein, hepatocytes normal configuration. The liver of Mice treated with (1/10 the LD50) of dimethoate showed that there were liver congestion and infiltration, vasodilation and congestion, necrosis. In Fig. 3 Hepatocytes in well architecture, sinusoids dilated in G3A groups. In the group treated with high dose (1/5 the. LD50) of dimethoate showed Infiltration and congestion, liver hemorrhage (Fig.4) in G4A group. In G5A group treated with Dimethoate and ZZLN leaf extract showed that Well architecture of hepatocytes, very less vasodilatation, Distinct nuclei (Fig 5).

Histopathological Alterations in kidney
Histopathological examination of the photomicrographs of the kidney sections in the control group showed a renal corpuscle and renal tubules, proximal convoluted tubules and distal convoluted tubules the Glomerulus, urinary space and Bowman's capsule were noticed as shown in group G1A (Fig. 6a). The histopathological examination slides of the kidney tissues in mice treated with dose of dimethoate, (15mg/kg bw) resulted in Histological changes in the Kidney including Glomerular Degeneration, Tubular Degeneration, Hemorrhage, Infiltration, Hydropic Changes, Tubular Cast, Tubular Widened Lumen and Glomerular Shrinkage, G2A (fig 7a). In the group treated with Dimethoate +ZZLN showed that recover the Glomerular degeneration, tubular degeneration and compressed blood vessals in G3A (fig 8a). The mice group treated with 1/5 of LD_{50} of Dimethoate showed shrinkage in glomerulus, Tubular Degeneration, Hemorrhage, Infiltration in G4A (fig 9a). Treatment of ZZLN leaf extract recover the Glomerular degeneration, tubular degeneration and compressed blood vessals in G5A (fig 10a).

CONCLUSION

The present findings clearly demonstrate that dimethoate is capable of inducing dose dependent histopathological changes in the liver and kidney of the exposed mice. According to these results, it is suggested that systemic insecticide like dimethoate exposure might cause hazardous effects, especially at high doses, to man and environment.
Organophosphate insecticides are known to induce various histopathological changes in the liver tissues .The acute and sub-chronic exposure to Dimethoate alters the antioxidant status and the histology of liver and induce hepatic lipid peroxidation in mice (Sivapiriya *et al*, 2006).

According to Choudhary *et al.,* (2003) the treatment with endosulfan, 10 mg/ kg/day in rats causes liver damage which includes dilation of sinusoidal spaces with irregular nuclear shape, degenerative changes includes binucleated cells, hypertrophy of hepatocytes and lymphocytic infiltration in the central vein.

According to Lone et.al (2013) who clarified that Dimethoate caused liver lymphocytic infiltration congestion, nuclear death enlargement of hepatic sinusoids hepatocellular damage, degeneration of nuclei. This is due to change in cellular integrity and membranes permeability due to exposure to chemicals (ajani et.al 2011). Dimethoate lipophilic substances can interact with plasma membranes and damage the membranes, or necrosis, releases enzymes in the circulation. Muthuviveganandave et.al (2011) suggested that hemorrhage, inflammatory cell infiltration occurred in dimethoate treated rats. Dimethoate treated group show large haemorrhagic areas, lobulated glomeruli, congested blood vessels, degenerative changes and infiltration of inflammatory cells in kidneys. In the leaf extract of ZZLN the presence of phytoconstituents such as flavonoids, alkaloids, glycosides, pectin, polysaccharides, peptide alkaloids, saponins, sterols, tannins, sterols, triterpenoic acids, fatty acids, ziziphin N, O, P, Q and dodecaacetylprodelphinidin B3, which participate in the healing and ameliorative properties. The leaf extract of ZZLN overcome the deleterious effect of dimethoate.

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