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Original Research

Biochemical, histopathological and molecular alterations in albino mice as biomarkers for exposure to acetamiprid insecticide

Authors:
Duha Zeki Al-Swefee[1], Ahmed Jasim Mohammed[1] and Salah M. Al-Chalabi[2]

Institution:
1. Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

2. Biotechnology Researches Center, Al-Nahrain University, Baghdad, Iraq.

Corresponding author:
Duha Zeki Al-Swefee

ABSTRACT:

The present study aimed to investigate the effects of acetamiprid (ACMP) on biochemical, histological and molecular aspects of albino mice. Forty albino mice at the age of 6-8 weeks and average weight 25±5 g were divided into four groups each having 10 healthy mice. The first group was orally administrated with distilled water while the second, third and fourth groups were orally administrated with 10, 20 and 40 mg/kg of acetamiprid respectively, (0.1mL) daily for six week. LD_{50} of acetamiprid was measured and found to be 200mg/kg. The parameters of biochemical evaluations included liver function by analyzing Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP). Lipid profile was analyzed through total cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL). Antioxidant factors that Superoxide Dismutase (SOD), Malondialdehyde (MDA), Catalase (CAT) and Glutathione Peroxidase (GPx). Liver and kidney tissues were taken as markers for histopathology, and % tail DNA as a marker for DNA damage. The results of biochemical parameters have shown significant differences ($P<0.01$) between control and acetamiprid concentrations. The lipids and liver function enzymes were increased as compared with control. MDA values recorded significantly increased compared to the control while SOD, CAT and GPx were decreased compared with the control. There are some alterations in tissues, also comet assay was a marvelous tool to assess the potential genotoxicity of acetamiprid. The study suggested that acetamiprid 40 mg/kg significantly affected the albino mice.

Keywords:

Acetamiprid, Cholesterol, Liver function, MDA, CAT, SOD, Comet assay, Kidney.

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INTRODUCTION

Our environment as well as our health are constantly threatened by various pollutants such as xenobiotics. Pesticides are one of the common pollutant groups in the world, and they have important disadvantage such as toxicity (Speck-Planche *et al*., 2012). The continuous use of pesticide showed hazardous effects on the physiological function of various body systems (Singh *et al*., 2012). Since pesticides are toxic, they are possibly hazardous to humans, animals, other organisms, and the environment (Lorenz, 2006). The widespread use of pesticides in arable regions all over the world have a threat to all living creatures and environments due to their toxic effects and accumulation of their hazardous features. Furthermore, pesticides spread fast through many agents such as water, air, and food chain (Rasgele *et al*., 2015). Insecticides are chemicals used to combat insects by killing them or preventing them from destructive cash crops (EPA, 2016). Insecticides are used in numerous formulations and delivery systems (eg., sprays, baits, slow-release diffusion) that effect their transport and chemical transformation. Mobilization of insecticides can occur round runoff (either dissolved or absorbed to soil particles), atmospheric deposition (primarily spray drift), or sub-surface flow (Goring and Hamaker, 1972; Moore and Ramamoorthy, 1984). Administration of the neonicotinoid insecticide may increase the stimulation of alterations on the cholinergic system of rats and mice, producing biochemical and behavioural effects that can be correlated to the toxicity produced by other kinds of pesticides that are linked to the development of neurodegenerative diseases such as Alzheimer's type dementia (Rodrigues *et al*., 2010). A biomarker is defined by (McCarthy and Shugart, 1990) the "measurements of body fluid, cells or tissues that indicate in biochemical or cellular in terms of the presence of contaminants or the magnitude of the host response". Furthermore there are many studies which indicated the increased use of pesticide resulted in toxicity in different species and could affect various functions like neurological, hematological, biochemical and reproductive function etc. in the body. These studies on toxicological aspect of insecticides are always useful for the rational treatment and prediction of risk of toxicity (Mondal *et al*., 2009). The comet assay, which is also referred to as the Single Cell Gel Electrophoresis assay (SCG or SCGE assay), is a rapid and quantitative technique by comet assay, evidence of DNA damage in eukaryotic cells could be noticed. It is founded on quantification of the denatured DNA fragments migrating out of the cell nucleus during electrophoresis. This assay has gained widespread expansion use in various aspects including human biomonitoring, genotoxicology, ecological monitoring and as a tool for research into DNA damage or repair in different cell types in response to a range of DNA-damaging agents (Collins, 2004). Thus in the present study we used mice to focused on some biochemical, histological and molecular changes as an indirect exposure in human and mammals in general.

MATERIALS AND METHODS

Animals

Fourty albino mice at the age of 6-8 weeks and average weight 25±5 g were obtained from Al-Razi center with good occupational safety and health in Baghdad. The mice were housed in polypropylene cages under controlled conditions of temperature 25±5°C and 12±2 h light/dark cycles. Diet and drinking water were given *ad libitum*. The animals were reared and treated in the animal house of Al-Nahrain university/ Biotechnology research center.

Chemical product

Commercial product of ACMP (Aster 20 SL, consisting of 200 g/L ACMP as active ingredient) is manufactured by Agrichem, Australia.

Determination of the median lethal dose (LD_{50})

The method used is divided into stages, with the outcome from each stage determine the next step to take

(*i.e.* whether to terminate or proceed to the next stage) and continued for 24 h (Chinedu *et al*., 2013).

Experimental design

The intragastric ACMP doses were selected according to the acute oral LD_{50} value of ACMP to be 200 mg/kg in albino mice. The body weight of controls as well as treated mice were taken weekly throughout the experiment and then on the day of sacrifice. For the preparation, dosage of each solution administrated was daily freshly prepared and adjusted weekly for body weight changes.

Treated groups

Acetamiprid was dissolved in the distilled water of pharmaceutical quality, and we have proceeded to give intragastrically 0.1mL of insecticide every day for seven days. The test concentrations were calculated depending on the percentage of active ingredients of commercial formulation of acetamiprid.

Group 1: 1.5 mL of acetamiprid 20 SL was dissolved in 28.5 mL of distilled water and administered at a dose of 1/20 of LD_{50} (10 mg/kg).

Group 2: 3 mL of acetamiprid was dissolved in 27 mL of distilled water and administered at a dose of 1/10 of LD_{50} (20 mg/kg).

Group 3: 6 mL of acetamiprid was dissolved in 24 mL of distilled water and administered at a dose of 1/5 of LD_{50} (40 mg/kg).

Control group: It received orally an equivalent volume of distilled water as previously described for treated groups.

Biochemical biomarkers

At the end of experiment period, blood sample was collected by cardiac puncture from each mice for hematological studies while plasma was extracted by centrifugation of the whole blood at 3000×g for 15 min for further biochemical analysis. Blood samples were collected in Eppendorf centrifuge tube and centrifuged. For further biochemical analysis, AST, ALT, ALP, TC, TG, HDL, LDL and VLDL were found out. Antioxidant factors such as SOD, MDA, CAT and GPx were also analysed.

AST level

The serum aspartate aminotransferase activity in the sample was determined according to the method of Reitman's and Frankel (1957) by the kit purchased from Spinreact company (Spain). AST activity was measured spectrophotometrically at the wave length of 340 nm.

ALT level

The serum alanine aminotransferase activity in the sample was determined according to the method of Reitman's and Frankel (1957) by the kits obtained from Spinreact company (Spain). ALT activity was measured spectrophotometrically at the wave length of 340 nm.

ALP level

Alkaline phosphatase was determined by the method reported by Belfield and Goldberg (1971) using ALP-kit obtained from Spinreact Company (Spain).

TC level

The method uses Tinder's (1969) colour system of peroxidase / phenol/ 4-amino antipyrine. The intensity of the red colour produced is directly proportional to the total cholesterol in the sample when read spectrophotometrically at the wave length of 500 nm.

TG level

The total concentration of triglycerides was measured by the enzymatic method reported by Fossati and Prencipe (1982) using commercially available kit (HUMAN Germany). The intensity of the produced color is read spectrophotometrically at 500 nm and it's directly proportional to the concentration of triglycerides in the sample.

HDL level

HDL Concentration was measured by enzymatic method (Gordon *et al*., 1977) with the commercially available kit (Spinreact company, Spain).

LDL level

The low density lipoprotein is determined according to modified Friedewald equation (Hata and

Nakajima, 1986).

LDL-C =T - Chol - (HDL-C + VLDL-C).

VLDL level

Very low density lipoprotein (mg/dl) was determined according to the conventional equation (Friedewald *et al*., 1972).

VLDL-C = TG / 5.

SOD activity

Abcam's superoxide dismutase activity assay kit (colorimetric) is a sensitive kit, where SOD activity was determined at the absorbance of 450 nm (Burton, 1956).

MDA level

The level of malondialdehyde was determined by a modified procedure described by Guidet and Shah (1989). MDA was measured at the wave length of 532 nm.

CAT activity

Bioassay Systems' improved assay directly measures catalase degradation of H_2O_2 using a redox dye. The change in color intensity at 570 nm or fluorescence intensity (λ em/ex = 585/530nm) was directly proportional to the catalase activity in the sample (Sinha, 1972).

GPx activity

Measurement of Serum GPx was done by using a sandwich enzyme immunoassay Elisa kit measured spectrophotometrically at a wavelength of 450 nm. The concentration of GPx in the samples was then determined by comparing the OD (Optical Density) of the samples to the standard curve (Moss and Henderson, 1999).

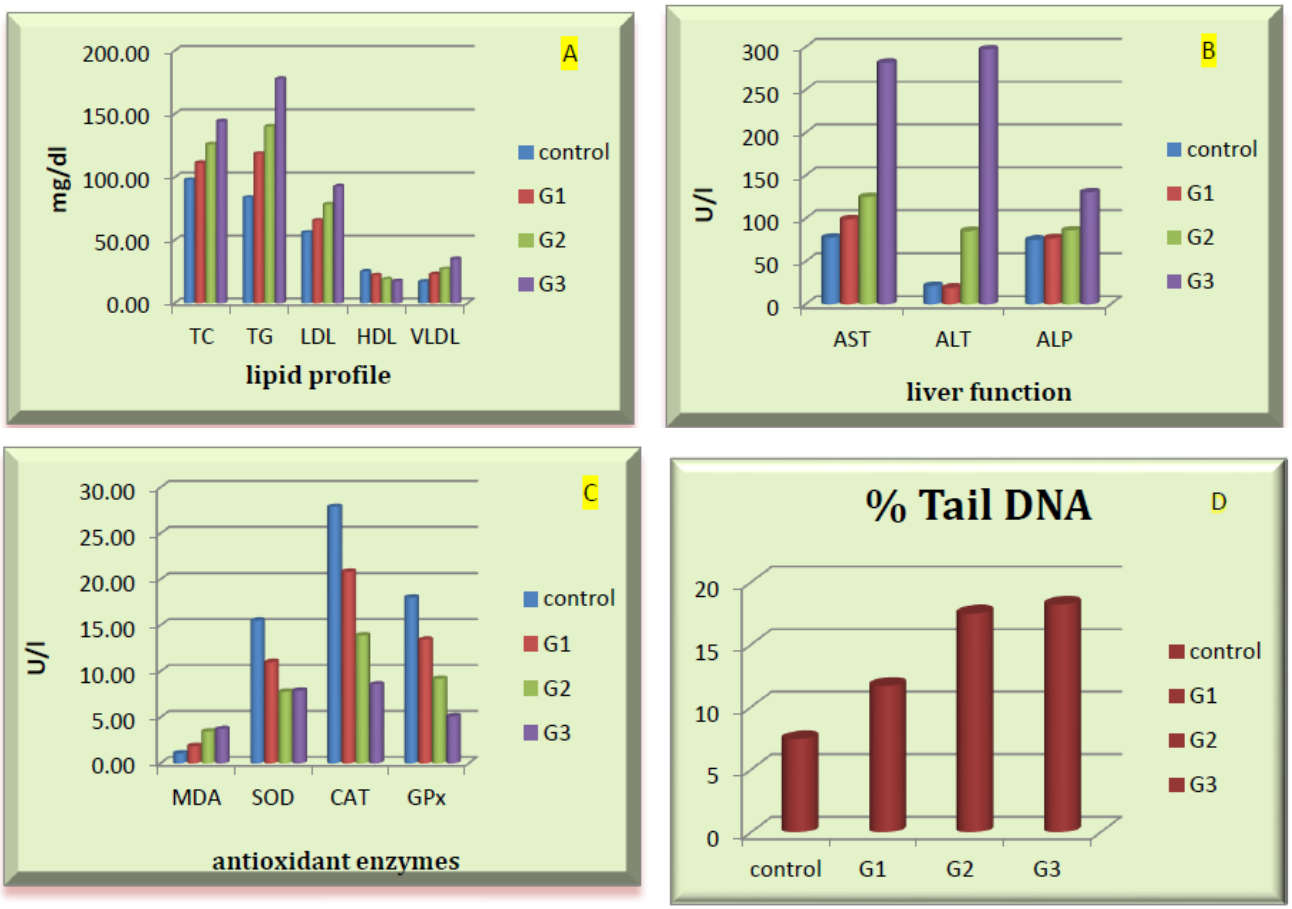

Figure 1. A. Means of lipid profile; B: Means of liver function with concentration exposed to acetamiprid subchronic concentrations with control sample; C: Means of antioxidant factor; D: Percentage (%) tail DNA with concentrations exposed to acetamiprid subchronic concentrations with control sample.

DNA damage

The procedure of comet assay was according to Singh *et al*. (1988); Kumari *et al*. (2008) for DNA single-strand breaks measuring with the Comet assay. DNA damage was quantified by measuring percentage (%) DNA in tail using computerized image analysis system (TriTek Comet- Score™ Freeware v1.5). Fifty cells were scored per slide, and geometric means were used to describe the damage.

Histopathological assessment

After subchronic exposure, mice were dissected, liver and kidney were quickly removed for the histopathological examinations. The tissue sections were prepared according to the method described by Bancroft and Gamble, (2008).

Statistical analysis

Statistical significance was determined using one-way analysis of variance (ANOVA) and least significant differences (LSD) were used to explain the differences between means at (P<0.01), the results were express as mean ± SEM (Gerry and Michael, 2002)

RESULTS

Effect of treatment on lipid profile and liver function enzymes

Total Cholesterol (TC)

There seen a high significant (P<0.01) increase in the level of serum cholesterol in animals groups treated with acetamiprid at 10, 20 and 40 mg/kg (111.33± 2.85, 126.00±2.89 and 144. 33± 2.40 mg/dl) respectively as compared with the control (98.00±0.58mg/dl) (Figure 1).

Triglyceride

The level of triglyceride in acute treatment obtained highly significant (P<0.01) increase in animals which treated with acetamiprid at 10, 20 and 40 mg/kg (118.67 ±1.20, 140.00±0.58 and 233.33± 9.02 mg/dl) respectively as compared with the control group (84.00±1.73 mg/dl) (Figure 1).

High, low and VLDL density lipoprotein: The results revealed a highly significant difference (P<0.01) decrease in HDL-C level in the acetamiprid treated groups at 10, 20 and 40 mg/kg (22.33±0.67, 19.00±0.58 and 17.33±0.88 mg/dl) respectively when compared with the control group (25.00±0.58 mg/dl).

The results of Figure 1 illustrated that there was a highly significant (P<0.01) increase in LDL-C level in the acetamiprid treated groups at 10, 20 and 40 mg/kg (65.67±2.67, 78.33±1.67 and 92.67±3.28 mg/dl) compared with control group (56.00±1.15 mg/dl). Finally, mean level of VLDL showed a highly significant (P<0.01) increase in groups treated with acetamiprid at 10, 20 and 40 mg/kg (23.00±0.58, 27.00±0.58 and 46.67± 1.80 mg/dl) compared with control group (17.00±0.58 mg/dl).

Aspartate aminotransferase (AST)

The results revealed high significant (P<0.01) increase in AST level in groups that treated with acetamiprid at 10, 20 and 40 mg/kg (99.00±1.15, 125.00±2.65 and 281.67±3.28 U/L) when compared with control group (77.67±1.20 U/L).

Table 2. Mean± SEM of antioxidant factors parameters of albino mice at six weeks after the intragastric administration of acetamiprid

S. No	Concentrations	MDA	SOD	CAT	GPx	Tail DNA %
1	Control: 0 mg/kg	1.06^{b}±0.04	15.50^{a}±0.76	27.87^{a}±0.44	18.00^{a}±0.45	7.44^{c}±1.70
2	Control: 1:10 mg/kg	1.86^{b}±0.08	10.97^{b}±0.33	20.80^{b}±0.52	13.43^{b}±0.32	11.70^{b}±1.25
3	Control: 2:20 mg/kg	3.43^{a}±0.22	7.73^{c}±0.23	13.87^{c}±0.22	9.17^{c}±0.27	17.50^{a}±0.83
4	Control: 3:40 mg/kg	3.73^{a}±0.09	7.87^{c}±0.12	5.43^{d}±0.41	5.10^{d}±0.06	18.20^{a}±2.9
5	LSD P≤0.05	0.22	0.74	0.65	0.53	4.55

Note: Small letters indicates comparison in column, similar letters are non-significantly, differences between means at (P≤ 0.05), using (LSD test).

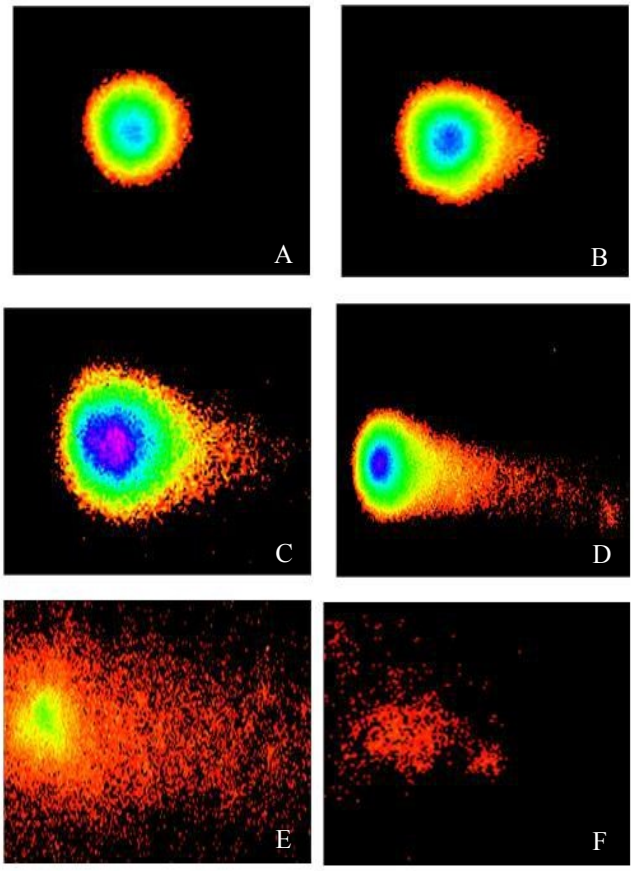

Figure 2: Representative comet images of albino mice erythrocytes following exposure to acetamiprid for six weeks. These comets illustrate the visual scoring classification class 0 (A), class 1 (B&C), class 2 (D), class 3 (E) and class 4 (F) (Cells are stained with ethidium Bromide).

Alanine aminotransferase (ALT)

There seen a highly significant (P<0.01) difference in the level of ALT in animals groups treated with acetamiprid at 10, 20 and 40 mg/kg (19.33±2.40, 85.33±2.96 and 90.00±6.51 U/L) as compared with the control (21.33±1.20 U/L).

Alkaline phosphatase (ALP)

Mean level of ALP revealed highly significant (P<0.01) difference in groups treated with acetamiprid at 10, 20 and 40 mg/kg (77.00±2.52, 86.00±1.15 and 230.67±0.88 U/L) compared with control group (75.33±2.40 U/L).

Effect of treatment on antioxidant factors and DNA damage

The results of antioxidant factors and DNA damage were illustrated in Table 2 and Figure 2.

Malondialdehyde or estimation of lipid peroxidation (MDA)

The results revealed highly significant P=0.0000 (P<0.01) difference in MDA level in groups treated with acetamiprid at 10, 20 and 40 mg/kg (1.86±0.08, 3.43±0.22 and 3.73± 0.09 µM) respectively as compared with the control group (1.06±0.04 µM).

Superoxide dismutase (SOD)

There was highly significant (P<0.01) decrease in the level of SOD in acetamiprid treated groups at 10, 20 and 40 mg/kg (10.97±0.33, 7.73±0.23 and 7.87±0.12 U/L) as compared with the control group (15.50±0.76 U/L).

Catalase (CAT)

The level of CAT in acute treatment obtained highly significant (P<0.01) decrease in animals which were treated with acetamiprid at 10, 20 and 40 mg/kg (20.80±0.52, 13.87±0.22 and 5.43±0.41 U/L) respectively as compared with control group (27.87±0.44 U/L).

Glutathione Peroxidase (GPx)

Mean level of GPx revealed high significant (P<0.01) decrease in groups treated with acetamiprid at 10, 20 and 40 mg/kg (13.43±0.32, 9.17±0.27 and 5.10±0.06 U/L) compared with control group (18.00±0.45 U/L).

Percentage tail DNA was a marker for DNA damage. Table 2 shows the results after subchronic exposure to acetamiprid. Percentage (%) tail DNA revealed highly significant (P=0.005) (P<0.01) increase at high concentration as compared with control. Group 3 (40 mg/kg) showed high mean value (18.20±2.9). Figure 3 showed the comet image of mice erythrocyte and damage classes.

DISCUSSION

The neonicotinoids have unique physical and toxicological properties as compared to earlier classes

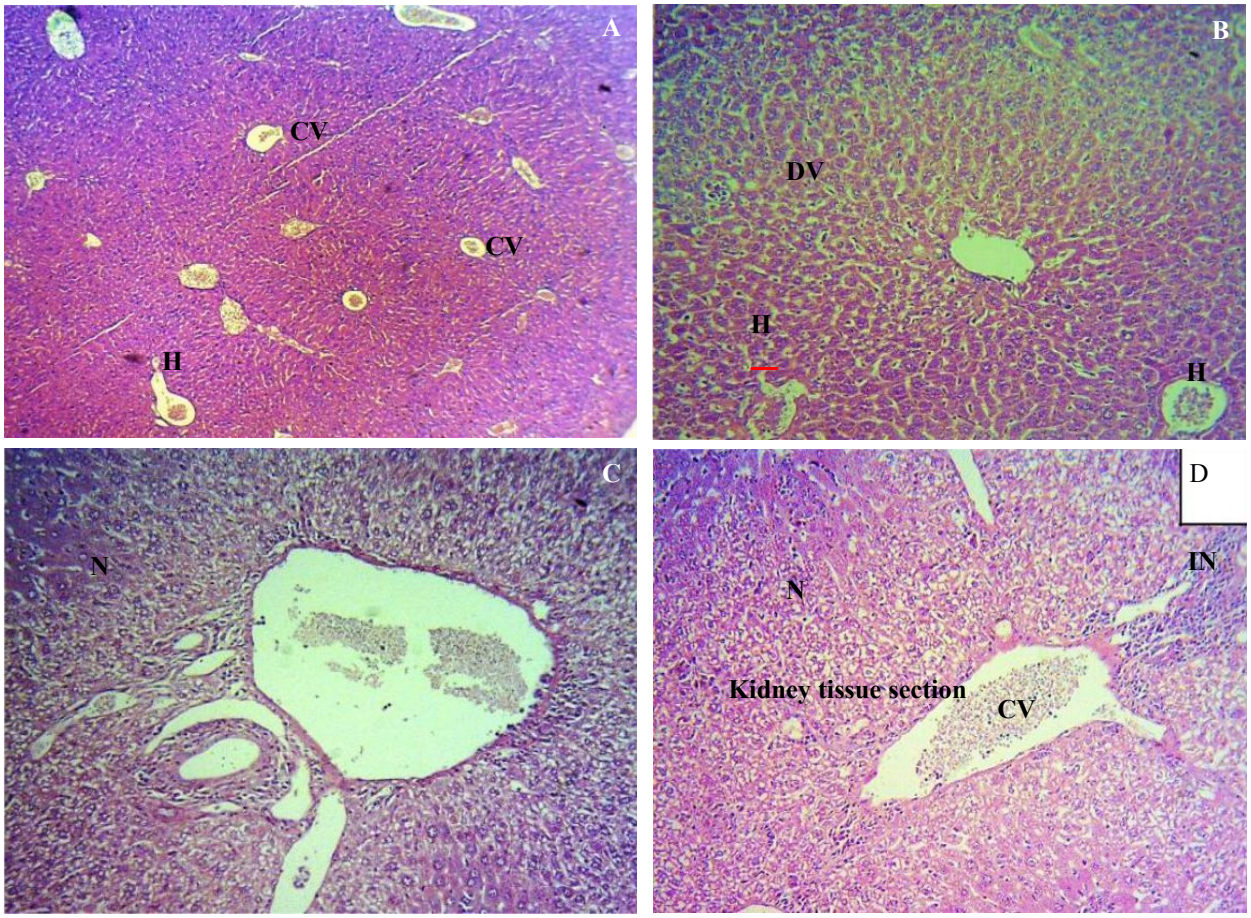

Figure 3. Effect of acetamiprid on the histological structure of the liver (10x H & E stain). Mice were given acetamiprid for six weeks and the histological structure of the liver was then examined with the use of hematoxylin and eosin staining. Photomicrographs showed the liver of mice from each treatment group: a) control showing normal appearance of hepatocyte (H) and central vein (cv); B) G_1 showing lack of normal pattern of hepatic cords; C) G_2 showing sever dilatation and congestion of central vein (DV) and hepatocyte necrosis (N); D) G_3 showing hepatocyte necrosis (N) and congested dilatation of central vein (CV) with infiltration of mononuclear leukocytes (IN).

of organic insecticides. The mammalian toxicity to neonicotinoids is considered to be centrally mediated because the symptoms of poisoning are similar to those of nicotine (Tomizawa and Casida, 2005). However, information on its toxicity to mammalian is scarce. The present study reveals statistically significant changes of some biochemical parameters of mice treated with the highest dose of acetamiprid. LD_{50} value was 200mg/kg that correlated with Gathwan *et al*. (2016) that the study of histological and cytogenetic effects of acetamiprid on male albino mice and The LD_{50} value of acetamiprid was reported to be 198 mg/ kg (approx.200 mg/kg) in male mice and 184 mg/kg in female mice (Singh *et al*., 2012).The biochemical findings showed a significant increase in lipid levels that include TC, TG, LDL, VLDL while HDL was decreased relatively to the control group (Figure 1A.).The increase in cholesterol value can also result from the inability of the organism to utilize or break it down to its derivatives or other useful products as a result of the toxicant effect (Edori *et al*., 2013). The elevation in serum total lipids and total cholesterol may be attributed to the stimulation of catecholamines, which stimulate lipolysis and increase fatty acid production. The elevation in total serum cholesterol level that observed in present investigation could be due to the block of liver bile ducts causing reduction or ces-

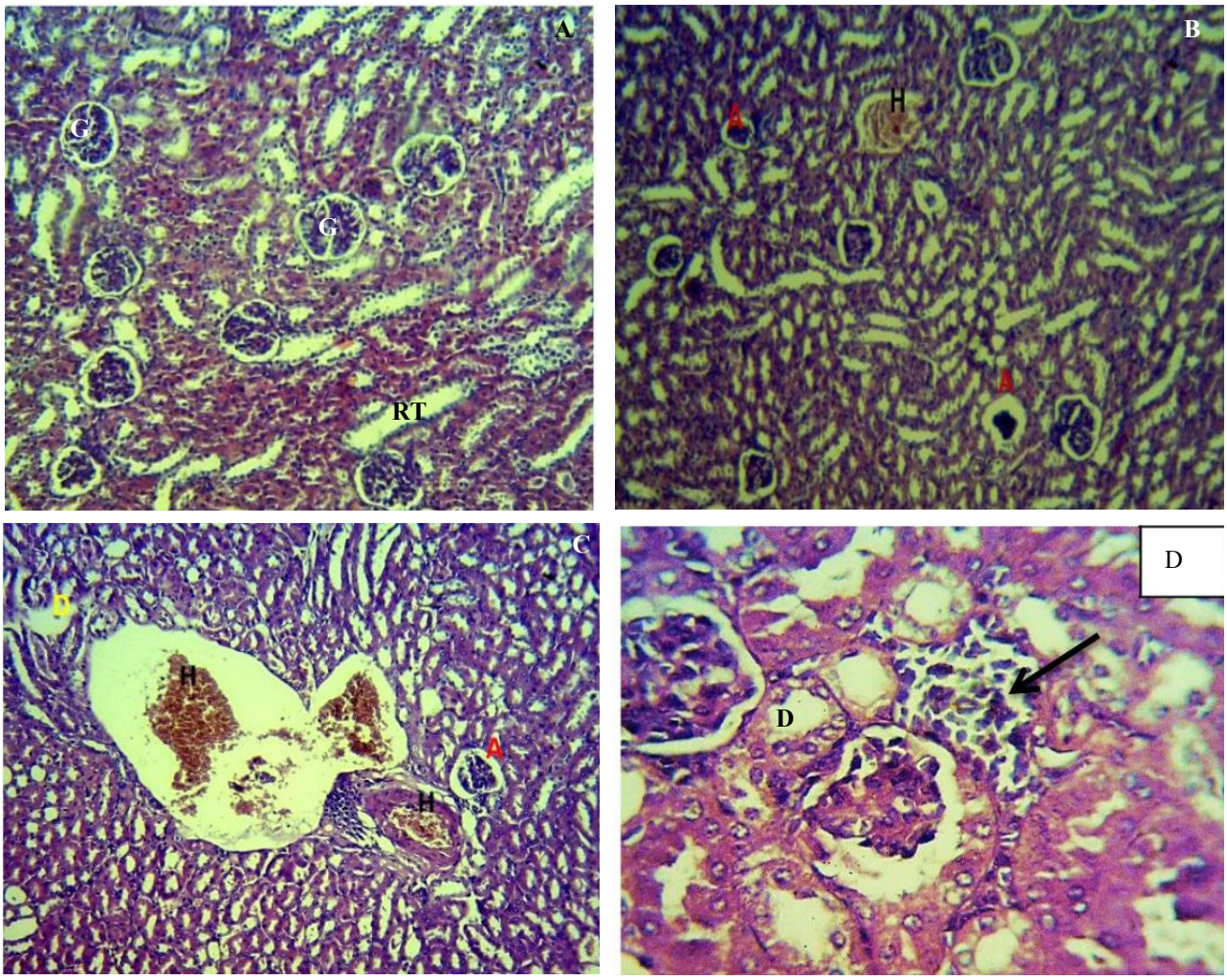

Figure 4. Effect of acetamiprid on the histological structure of the kidneys (40x H and E stain). Mice were given acetamiprid for 6 weeks and the histological structure of the kidney was then examined with the use of hematoxylin and eosin staining. Photomicrographs show the kidneys of mice from each treatment group: A). Control, normal appearance of glomerulus (G) and renal tubule (RT); B) G_1 showing congested dilated blood vessels between renal tubules (H) and atrophy of glomerulus (A); C) G_2 showing heamorrhage (H) in renal tubules with degeneration (D) and atrophy of glomerular (A); D) G_3 showing aggregation of lymphocytes, degeneration of dilated tubules (D).

sation of its secretion to the duodenum subsequently causing cholestasis (Rai *et al*., 2009). The findings in the present study correlated with the findings of Mondal (2007) in oral administration of acetamiprid to female wister rats. Zhang *et al*. (2010) confirmed significant elevation in the activity of serum Alanine Transaminase (ALT) of male mice in acetamiprid toxicity. Bhardwaj *et al*. (2010) reported elevation in ALT in imidacloprid toxicity in female rats. The present findings of elevation in the value of AST (Figure 1B.) was in agreement with the findings of Bhardwaj *et al*. (2010) in female rats following orally administration of imidacloprid and Zhang *et al*. (2010) in male mice following orally administrations of acetamiprid. Elevation in Alkaline phosphatase value was also reported by other workers such as acetamiprid toxicity in female rats (Mondal *et al*., 2009) and acetamiprid in male mice (Zhang *et al*., 2010). The increase in ALP usually occurred due to its increased synthesis due to damaged liver conditions (Seetharam *et al*., 1986). Elevated plasma ALP might be due to acute hepatocellular damage and destruction of epithelial cells in gastrointestinal tracts (Zimmerman,

1969). The results of antioxidant factor showed that increase in MDA while SOD, CAT and GPx were decreased (Figure 1C). MDA is a biomarker that provides an indication of lipid peroxidation level (Bhale *et al*., 2014). The most harmful impact of oxidative stress is lipid peroxidation, which has been implicated in the pathogenesis of numerous diseases including atherosclerosis, diabetes, cancer, and aging (Spiteller, 2007). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are the first line of defence against ROS and other free radicals (Rahaman *et al*., 1999). Whenever the amount of ROS and other radicals increase the capacity of such enzymes, non-detoxified radicals that attack cellular macromolecules. Increase in production of radicals may found in cases of diseases, as well as during the biotransformation of various xenobiotics (Mao *et al*., 2007). Kapoor *et al*. (2010) indicated that exposure to high doses of imidacloprid (20 mg/kg/day) for 90 days produced significant decreases in Superoxide Dismutase (SOD), catalase (CAT), and Glutathione Peroxidase (GPx) activities in the brains of female rats. Pesticides may induce oxidative stress, leading to the generation of free radicals and causing lipid peroxidation, and may cause molecular mechanism that gives rise to pesticide induced toxicity (Ismail and Mohamed, 2012; Rheim *et al*., 2015). Excessive production of oxidants can result in oxidative damage, due to the oxidation of lipids, proteins and DNA (Figure 1D. and Figure 2). There is increasing evidence that oxidative stress, particularly stress caused by reactive oxygen species and reactive nitrogen species, can lead to numerous inflammatory degenerative diseases (MacDonald-Wicks *et al*., 2006). The results of kidney and liver tissue section shown in Figures (3 and 4). Gathwan *et al*. (2016) who reported histopathological changes in liver that congestion and mild hemorrhages, degeneration in the hepatocytes and dilation of the hepatic sinusoids, pyknotic or karyolytic nuclei in hepatocytes, pale stain in the cytoplasm of the hepatocytes, vacuoles in the cytoplasm of the hepatocytes, focal necrosis of hepatocytes in the portal and periportal areas, red pulp congested with red blood cells and hemorrhagic areas. The previous experiment has found that acetamiprid increased the levels of AST, ALT and ALP (Zhang *et al*., 2011) which gave further evidence that the detrimental effects of acetamiprid on the kidney were mediated by its metabolite.

CONCLUSION

In this study, acetamiprid has deleterious effects on kidney and liver potentially through the oxidative stress of its metabolites also the harmful effect on lipids thus, acetamiprid should be used in a restricted and careful manner to protect mammalian and other non-target animals. Comet assay was a good tool to assess the genotoxicity, it can be concluded that antioxidant system that include (SOD, CAT, GPx and MDA) were effective biomarkers for evaluating the toxicity of insecticide (acetamiprid) in mice and subsequently to human.

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