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Research Article

Protective Effects of Manuka Honey Against Diazinon Induced Hepatorenal Toxicity in Adult Male Rats

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Abstract

Manuka honey is mono-floral honey produced by Manuka myrtle tree. MH has anti-inflammatory, anti-bacterial, anticancer, wound healing and other benefits. This experimental study aimed to monitor Diazinon administration effects on liver and kidney of adult male Albino rats and protective value of 6 weeks administration of MH against effects of Diazinon and mechanism of their actions. Forty rats weighing 180-220 g divided into four groups (10 rats each): Group I (control group): rats received 0.2 mg/kg corn oil, daily for 6 weeks. Group II (DZN group): rats orally administrated 50 mg/kg DZN in corn oil, daily for 6 weeks. Group III (MH group): rats orally administrated 1.5 g/kg MH daily for 6 weeks. Group IV (DZN + MH group): rats orally administrated 50 mg/kg DZN and 1.5 g/kg MH, daily for 6 weeks. After six weeks, blood samples collected from retro-orbital venous plexus. Serum samples used to determine levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin, creatinine, blood urea nitrogen (BUN), total protein (TP), albumin (ALB), glucose, triglycerides (TG), total cholesterol (CHO), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) very-low-density lipoprotein cholesterol (VLDL-c), creatinine kinase (CK) and lactate dehydrogenase (LDH). After blood sampling, livers and kidneys isolated, fixed in 10% buffered formalin and examined under light microscope. DZN led to hyperglycemia, increased in serum levels of triglyceride, CHO, LDL-C, VLDL-C, AST, ALT, ALP, GGT, creatinine, Blood urea nitrogen, CK and LDH but significant decreased in total proteins and albumin. Administration of MH led to improvement of blood glucose, lipid profile, liver function tests and kidney function tests, Tissue destructive enzymes. These improvements were confirmed histologically by examination of hepatic and renal tissues. In conclusions, DZN had hepatotoxicity and renal toxicity that improved by Manuka honey administration for 6 weeks.

Keywords: Manuka Honey; Diazinon; Physiology; Histology; Kidney; Liver; Rats

Introduction

Pesticides are highly poisonous organic chemical compounds that are introduced into the environment to control crop pests and disease vectors. Pesticides can be harmful to one's health, especially if one exposed to them on a frequent basis (e.g., farmers, field workers, etc.). Most farmers in developing nations are less educated and are unaware of or have never been instructed on how to properly handle chemicals [1]. Pesticides containing organophosphates (OPs) have progressively increased in use around the world. OPs cause genotoxicity, neurotoxicity, cardiotoxicity, immunotoxicity, hepatotoxicity, nephrotoxicity, reproductive toxicity and metabolic problems in both humans and animals [2]. One of the most extensively used OPs in agriculture is

Diazinon (DZN) [3]. Association between DZN and hyperglycemia was reported [4]. DZN poisoning inhibited insulin secretion by disrupting glutamate dehydrogenase activity in pancreatic Langerhans islets and also by block insulin release from the pancreas, resulting in hyperglycemia [5]. Recent studies have been carried out to evaluate the potential role of antioxidants for the protection of cells against organs damage from environmental pollutants. Of these compound is Manuka Honey [6], which act as an antioxidant. Manuka honey is a mono-floral honey produced by Manuka myrtle tree (*Leptospermum scoparium*), which grows primarily in New Zealand and Eastern Australia as a shrub or small tree [7]. The health benefits of Manuka honey are well known and are related to its unique chemical composition [8]. Manuka honey has anti-inflammatory, antibacterial, anticancer, wound healing and other benefits [7].

The goal of the present study was to assess the protective effects of Manuka Honey administration for 6 weeks on hepatorenal toxicity induced by Diazinon in adult male Albino rats.

Material and Methods

Materials

The insecticide Diazinon (O, O-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphonothioate) and the medically graded Manuka honey (UMF18+) were purchased from local market in Jeddah, Saudi Arabia.

Animals

Forty adult male albino rats of Wistar strain (*Rattus norvegicus*) weighing 180-220g were used in this experimental study. Rats were housed in standard plastic cages and maintained under controlled laboratory conditions of humidity (65%), temperature (25±1°C) and 12:12 h light: dark cycle. Rats were feed on normal commercial chow and had free access to water *ad libitum*. Experimental treatments were done in accordance with the ethical guidelines of the Animal Care and Use Committee of King Abdulaziz University and according to the ARRIVE (Animals in Research: Reporting In Vivo Experiments) reporting guideline.

Methods

Experimental groups

The experiment was designed to carry out the treatments (orally) at the level of one of LD_{50} of diazinon (50 mg/kg) and plant oils. Forty rats were randomizing divided into four groups (10 rats each) as follows: Group 1 (control group): rats received 0.2 mg/kg corn oil, daily for 6 weeks. Group 2 (DZN group): rats orally administrated 50 mg/kg DZN in corn oil, daily for 6 weeks. Group 3 (MH group): rats orally administrated 1.5 g/kg MH daily for 6 weeks. Group 4 (DZN + MH group): rats orally administrated 50 mg/kg DZN and 1.5 g/kg MH, daily for 6 weeks.

Determination of LD_{50} of diazinon

Diazinon toxicity in rats was calculated to determine lethal and sub lethal doses LD_{50}, and it was found to be 600 mg/kg b.w.

Body weight determinations

The body weights of rats were determined at the start and at the end of the experimental period using a digital balance from (OHAUS, Model: Scout Pro SPU601, Made in China). The weight gain was determined by subtract final from initial body weight. Percentage change of body weight was determined by dividing weight gain by the initial body weight [9].

Blood serum analysis

After six weeks, the experimental animals were allowed to fast for 12hs, and then anesthetized with diethyl ether. Blood samples were collected from retro-orbital venous plexus in non-heparinized tubes, centrifuged at 2500 rpm for 15 min and blood sera were then collected and stored at -80°C till used.

Glucose concentration was determined using the method of [10]. Lipid profile was estimated in serum as triglycerides (TG) [11], total cholesterol [12], HDL-C [13]. The concentration of LDL-c was determined using Friedewald., *et al*. [14] equation [LDL-c = Total cholesterol - HDL- triglycerides/ 5]. Serum VLDL-c level was determined using the following equation = VLDL-c = Triglycerides/2.175. Serum samples were used to determine liver function by analysis of levels of alanine aminotransferase (ALT) [15], aspartate aminotransferase (AST) enzyme activity [15], alkaline phosphatase (ALP) enzyme activity [16], gamma-glutamyl transferase (GGT) [17], total bilirubin (TBIL) [18], total proteins [19] and albumin (ALB) [20]. Kidney function tests were determined as serum levels of creatinine (CR) [21] and blood urea nitrogen (BUN) [22]. Tissue destructive markers were determined as serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH) [23].

Statistics analysis

The data were analyzed using IBM SPSS Statistics for Windows, version 23 (IBM SPSS, IBM Corp., Armonk, N.Y., USA). Collected data presented as mean ± standard deviation (SD). Statistical comparisons were performed by One-Way analysis of variance followed by least significance difference (LSD) test for comparison between different groups. All statements of significance were based on probability.

Results

Body weights

The initial body weights were significantly decreased in DZN, Honey + DZN versus control ($P < 0.0001$, $P < 0.010$, respectively) but was significantly increased in Honey group versus DZN ($P < 0.0001$). The final body weights were significantly decreased in DZN group versus control and Honey groups ($P < 0.0001$ for both). Weight gain in Honey group was significantly decreased versus control ($P < 0.050$) but was significantly increased versus DZN group ($P < 0.050$) (Figure 1).

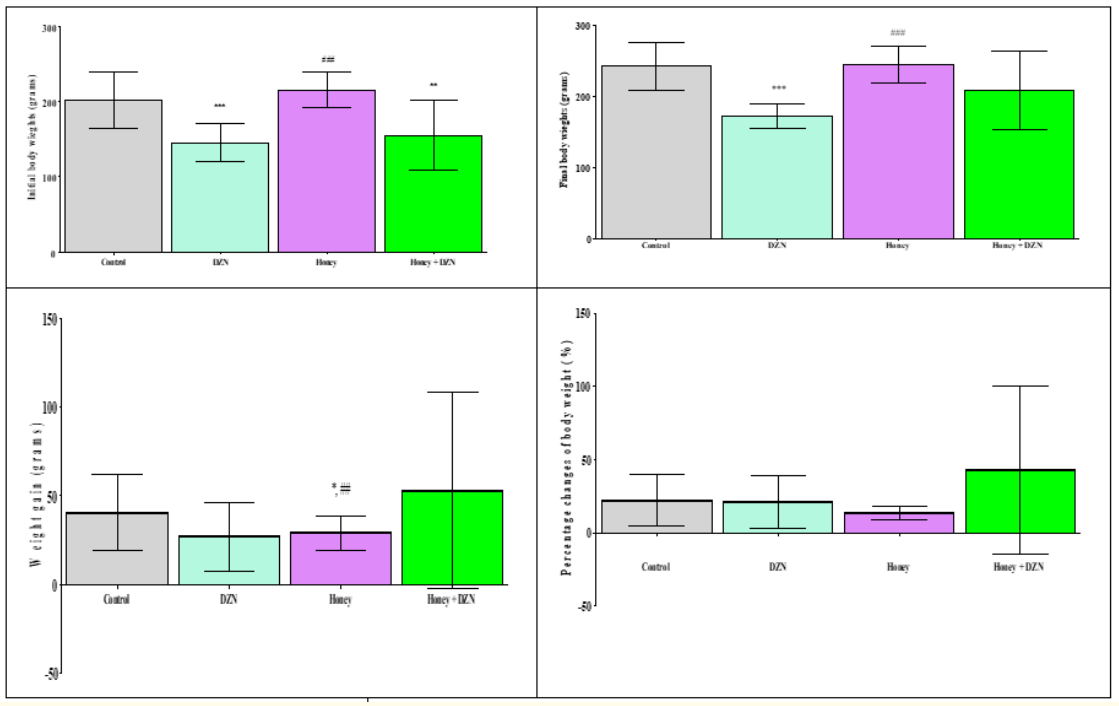

Figure 1: Initial body weights, final body weights, weight gains and percentage changes of body weights in different studied groups.

*: Significance versus control; #: Significance versus DZN group.

Blood glucose and serum levels of total proteins and albumin

The blood glucose levels were significantly decreased in Honey group versus DZN and Honey + DZN groups ($P < 0.0001$ for both). The serum total proteins and albumin levels were significantly decreased in DZN, Honey, Honey + DZN versus control ($P < 0.0001$ for all). Meanwhile, serum albumin levels were significantly increased in Honey and Honey + DZN groups versus DZN group ($P < 0.0001$ for both) (Figure 2).

Lipid profile serum levels

The serum levels of triglyceride were significantly increased in DZN, Honey and Honey+ DZN groups versus control ($P < 0.010$, $P < 0.050$, $P < 0.050$, respectively). The serum levels of total

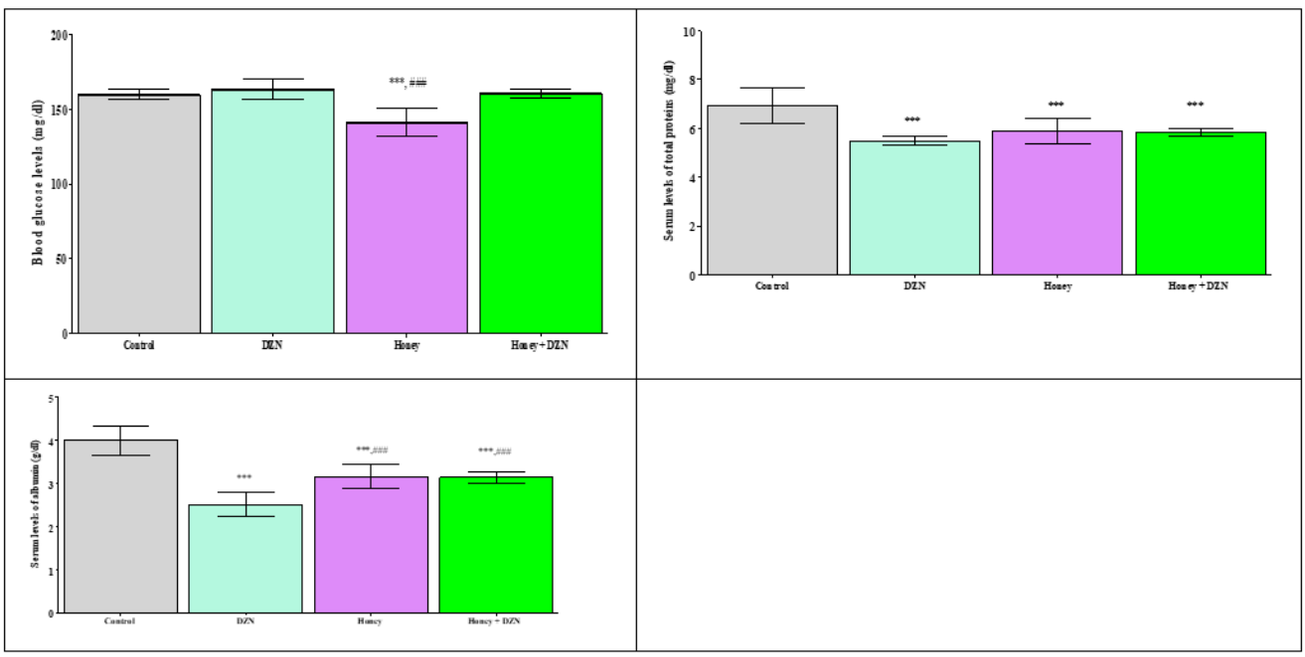

Figure 2: Levels of glucose (mg/dl), total proteins (mg/dl) and albumin (g/dl) in different studied groups. *: Significance versus control; #: Significance versus DZN group.

cholesterol, LDL-C and VLDL-C were significantly increased in DZN ($P < 0.0001$) and Honey+ DZN ($P < 0.050$) groups versus control but were significantly decreased in Honey, Honey+ DZN groups versus DZN group ($P < 0.0001$). The serum levels of HDL-C were significantly decreased in DZN, Honey, Honey+ DZN, groups versus control ($P < 0.0001$, $P < 0.050$, and $P < 0.0001$ respectively) but were significantly increased in Honey, Honey+ DZN groups versus DZN group ($P < 0.0001$ for all) (Figure 3).

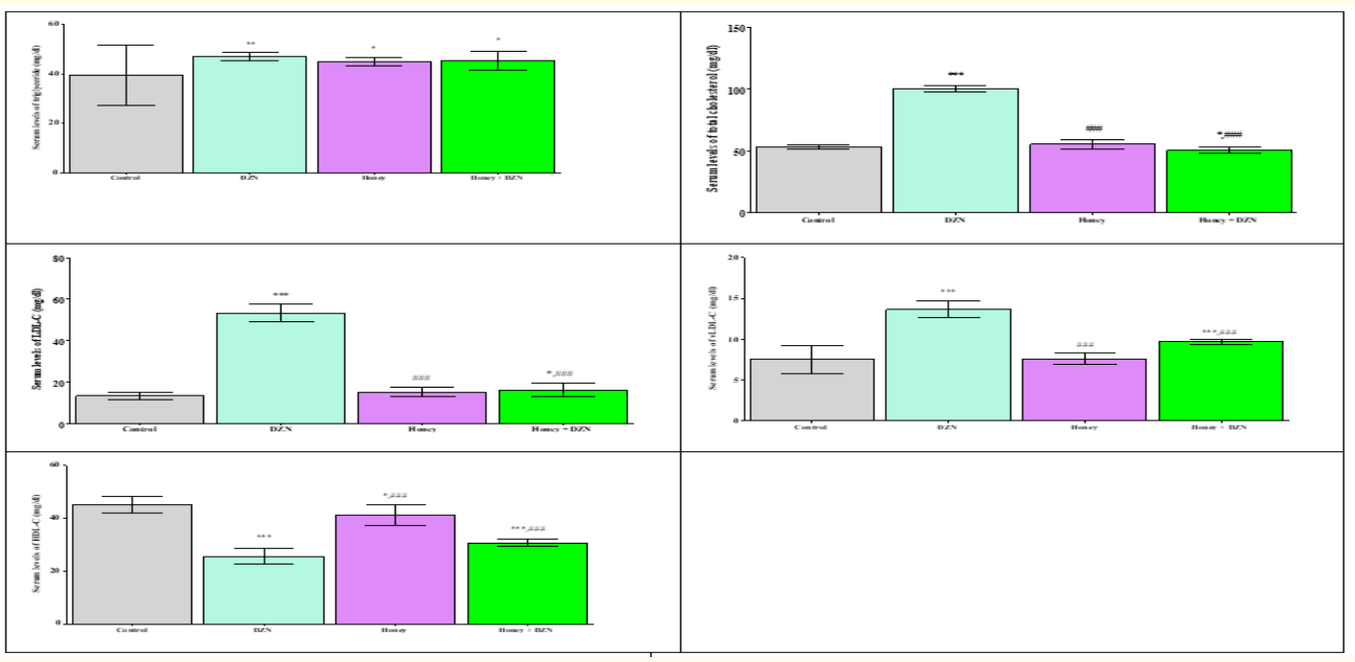

Figure 3: Levels of lipid profile in different studied groups.

*: Significance versus control; #: Significance versus DZN.

Liver function tests

The serum levels of ALT, AST, ALP and GGT were significantly increased in DZN, Honey+ DZN groups versus control (P < 0.0001); but were significantly decreased in Honey, Honey + DZN, versus DZN (P < 0.0001). Meanwhile, ALP and GGT serum levels were significantly decreased in Honey group versus control (P <0.0001 and P <0.050) (Figure 4).

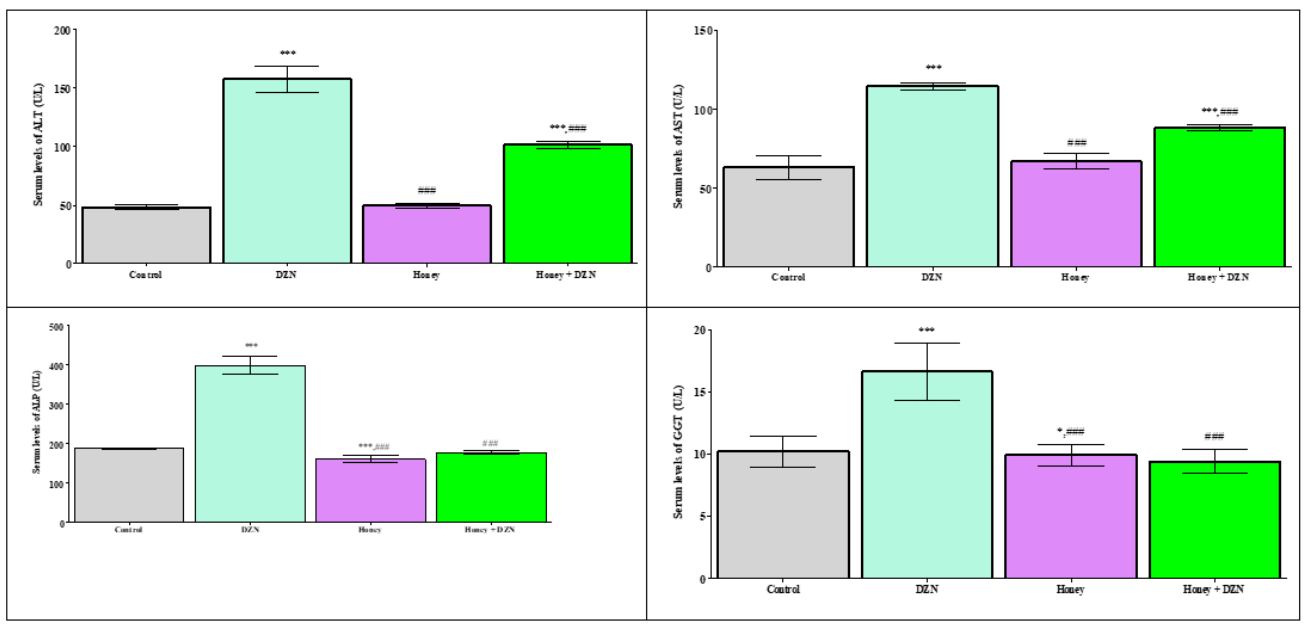

Figure 4: Liver function tests in different studied groups.

*: Significance versus control; #: Significance versus DZN.

Kidney function tests

The serum levels of creatinine and urea nitrogen were significantly decreased in control, Honey, and Honey+ DZN groups versus DZN (P < 0.0001, P < 0.010, P < 0.010, and P < 0.0001for all). Creatinine serum level was significantly decreased in Honey group versus control (P <0.010); while urea nitrogen serum level was significantly decreased in Honey + DZN versus control (P <0.0001) (Figure 5).

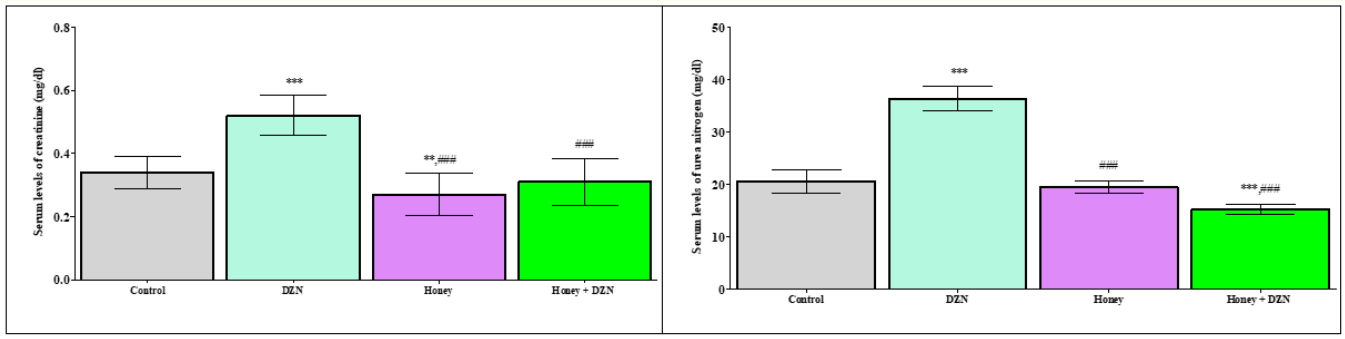

Figure 5: Kidney function tests in different studied groups.

*: Significance versus control; #: Significance versus DZN.

Tissue destructive enzymes levels

The serum levels of creatine kinase and lactate dehydrogenase were significantly increased in DZN ($P < 0.0001$ for both) and Honey+ DZN groups ($P < 0.050$ and $P < 0.0001$) versus control; but were significantly decreased in Honey and Honey + DZN versus DZN ($P < 0.0001$ for all) figure 6.

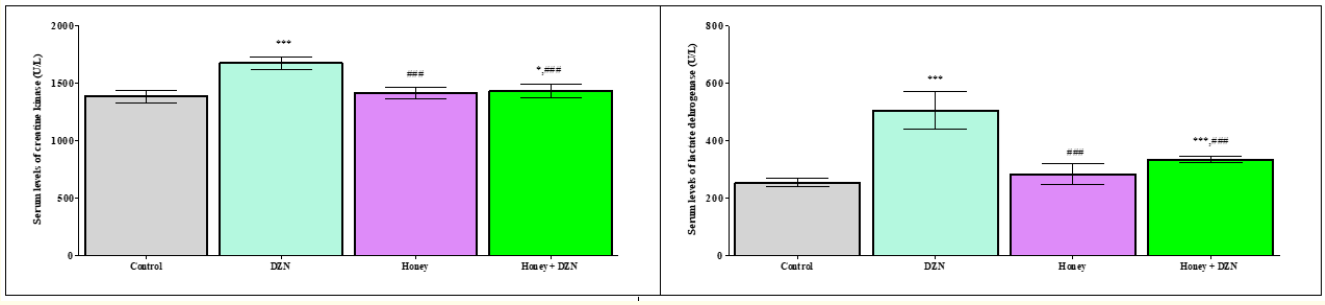

Figure 6: Serum levels of creatine kinase (U/L) and lactate dehydrogenase (U/L) in different studied groups.

*: Significance versus control; #: Significance versus DZN group.

Histological results

Histological results of liver

Liver of control rat showing normal histological structure of central vein, portal area and hepatic parenchymal cells (Figures 7 A&B). In DZN treated rats**,** portal area showed mild edema, few inflammatory cells infiltration around the bile duct, and congested portal vein, peri-ductal inflammatory cells infiltration with mild proliferation of bile duct epithelium and scattered hepatic cells with vacuolar degeneration, dilatation of the hepatic sinusoids and mild vacuolar degeneration of the hepatic cells and scattered necrosis (Figures 7 C&D). In Honey treated group, liver parenchyma showed near to normal appearance of the hepatic parenchyma of central vein and hepatic cells and mild vacuolar degeneration and necrosis, mild degenerative changes of hepatic cells with activated Kupffer cells with congestion of central veins (Figures 7 E&F). In Honey + DZN treated rats, liver of rats which administrated honey and toxin showed mild hepatocellular degenerative changes, few necrotic cells, and activated Kupffer cells, diffuse vacuolar degeneration of hepatic cells, some with pyknotic nuclei and scattered necrotic cells and dilated hepatic sinusoids (Figures 7 G&H).

Figures 7: A&B: Liver of control rat showing normal histological structure of central vein (CV) and hepatic parenchymal cells (arrows). C&D: Diazinon Treated rats: Portal area showed mild edema (arrow), few inflammatory cells infiltration around the bile duct (dashed arrow), and congested portal vein (short arrow). congestion of the central vein (CO), and mild vacuolar degeneration (arrow) of the hepatic cells with scattered necrotic cells (dashed arrow). E&F: Honey treated group: Liver parenchyma showed mild degenerative changes of the hepatic cells with activated Kupffer cells (arrow). Normal appearance of the hepatic parenchyma of central vein (CV) and hepatic cells (HCs). G&H: Diazinon balsam and honey treated rats showed some congested central veins (arrow, G) and apparently normal hepatic cells with few degenerated cells. H: Showed apparently normal hepatic cells, good protection of the hepatic parenchymal cell with few degenerated cells (dashed arrow) and activated Kupffer cells (arrow). H&E, (A, C&GX200), (B, D, E, F&H: X400).

Histological results of kidney

In Control rats, the kidney showed normal architecture with normal glomerulus and renal tubules with intact cell borders and had brush borders. No signs of abnormalities (Figure 8 A&B). In DZN treated rat group, most of kidney parenchyma showed glomerular degeneration and atrophy and leukocytic infiltration and congestion. Renal tubules were showed necrotic and degenerative changes with disrupted cell borders and eosinophilic cytoplasm. Congestion of blood capillaries in between degenerated tubules were present (Figures 8 C&D). In Honey treated rats, some of glomeruli showed signs of degeneration and necrosis and appeared small while others showed normal structure. Congestion of interstitial blood capillaries was also observed, and some tubules showed signs of degeneration with unremarkable cell borders (Figures 8 E&F). In Honey + DZN treated rats, the kidney showed degenerative and necrotic changes in glomeruli. The proximal and distal collecting tubules showed degenerative and necrotic changes with pale staining cytoplasm and disappearance of cell boundaries. Normal glomeruli were also observed in between the affected ones (Figures 8 G&H).

Figures 8: A&B: Control rats, the kidney showed normal architecture with normal glomerulus (arrows) and renal tubules (arrowheads). C&D: Diazinon treated rat groups. Most of the kidney parenchyma showed glomerular degeneration and atrophy and leukocytic infiltration (arrows), and congestion (double arrows). Renal tubules were severely damaged showed necrotic and degenerative changes with disrupted cell borders and eosinophilic cytoplasm (double arrowheads). E&F: Honey treated rats: some of the glomeruli are showed signs of degeneration and necrosis and appeared small (arrows). and others showed normal structure (double arrows). congestion of the interstitial blood capillaries was also observed (double arrowheads) and some tubules were showed signs of degeneration with unremarkable cell borders (arrowheads). G&H: Diazinon and Honey treated rats: The kidney showed degenerative and necrotic changes in the glomeruli (arrows, G). the proximal and distal collecting tubules showed degenerative and necrotic changes with pale staining cytoplasm and disappearance of the cell boundaries (double arrows). Normal glomeruli were also observed inbetween the affected ones (arrowheads, G and H). Hx&E (A, C, E&G: X100), B, D, F&H: X400).

Discussion and Conclusion

The purpose of the present study was to find out the protective effect of Manuka honey against the hepatorenal toxic adverse effects of the pesticide Diazinon in adult male albino rats. Thus, the present work evaluated the hazards of DZN on body weight weights, changes of liver and kidney functions, on blood glucose and lipid profile as well as tissue destruction markers as creatinine kinase and lactate dehydrogenase. Then, histopathological changes of liver and kidney tissues were studied using light microscopy.

The present study results revealed that initial body weights were significantly decreased in DZN, Honey + DZN versus control and but was significantly increased in Honey group versus DZN. The final body weights were significantly decreased in DZN group versus control and Honey groups. Weight gain in Honey group was significantly decreased versus control but was significantly increased versus DZN group. In experimental animals, OP insecticides promote body weight loss [24]. It was postulated that body weight of rats decreased after 4 and 7 weeks of DZN administration and that this decline was related to decreased food intake [25]. In another study, Mossa., *et al.* [26] stated that, body weights were significantly decreased in rats exposed to DZN. The decrease in body weight may be due to reduced food consumption of exposed-rats and also due to overall increased degradation of lipids and proteins as a result of the direct effects of organophosphate compound DZN [27]. Stromborg [28] reported that dietary levels of DZN above 50 mg/kg were associated with reduced food consumption, weight loss and reduction in egg production in northern bobwhites. In addition, others OPIs cause reduction of body weight in rats [24] and mice.

Changes in blood glucose, lipid profile, liver function and kidney function tests and tissue destructive enzymes were measured in all studied groups in the present study. These results revealed that, blood glucose levels were significantly decreased in Honey group versus DZN and Honey + DZN groups. Insignificant changes in blood glucose observed in the present study between control and DZN group were consistent with previous studies [29]. Meanwhile, Al-Attar and Abu Zeid [30] reported hyperglycemia in mice exposed to DZN. Exposure to DZN caused a severe disturbance of lipids, carbohydrates, and proteins metabolism. The causes of hyperglycemia may be due to enhancement of activities of enzymes involved in gluconeogenesis leading to the formation of glucose from non-carbohydrate sources in addition to suppression of hepatic glycogenolysis or stimulating glycogenolysis processes to increase blood glucose level from liver as an essential source of carbohydrates in body. Important causes of DZN toxicity include its ability to cause organ damage, alter cellular antioxidative capability and disrupt glucose homeostasis [31]. When islets of Langerhans are exposed to reactive agents, oxidative stress plays a key role. Antioxidant capability in pancreatic islets is basically decreased when oxidative damage is produced. As a result, any injury to pancreatic ß-cells has a high probability of causing hyperglycemia [32]. Interestingly, in the present study, when rats were treated with Honey significant decrease was observed in blood glucose levels versus DZN and DZN + Honey groups suggesting Honey is more effective in reducing blood glucose levels in rats treated with DZN that may be related to its antioxidant capacity.

The serum total proteins and albumin levels were significantly decreased in DZN, Honey, Honey + DZN versus control. Meanwhile, serum albumin levels were significantly increased in Honey and Honey + DZN groups versus DZN group. Previous studies reported that serum levels of total proteins and albumin, significant reductions were observed with DZN treated group versus control as reported previously [30,33,34]. DZN can reduce the total protein by decreasing its formation by the liver, resulting in reduction of total proteins and albumin in the blood [33]. On the other hand, DZN was reported to bind with albumin and reduce its activity resulting in a decrease in its levels in the blood [34]. The hypoproteinemia in the present study may be due to a decrease in protein formation and/or due to several pathological processes as liver injury, renal damage and increase in protein excretion in urine [35]. In the present study, none of the groups recovered total protein or albumin levels when administered honey.

In the present study, serum levels of triglyceride were significantly increased in DZN, Honey and Honey+ DZN groups versus control. The serum levels of total cholesterol; LDL-c and VLDL-c were significantly increased in DZN and Honey+ DZN groups versus control but were significantly decreased in Honey, Honey+ DZN groups versus DZN group. Meanwhile, serum levels of HDL-c were significantly decreased in DZN, Honey, Honey+ DZN, groups versus control but were significantly increased in Honey, Honey+

DZN groups versus DZN group. In this respect, significant increase in triglycerides in mice treated with DZN was reported, and also that DZN pesticide-induced hypercholesterolemia [30]. Generally, pesticides suppressed hepatic cytochrome P-450 enzyme. Cholesterol levels increase indicates inhibitory action of pesticide on cytochrome P-450 enzyme. Elevated cholesterol serum levels indicate liver disorders and cholestasis. Moreover, stagnation of bile flow in bile ducts caused by periportal cell damage (emphasis by raised ALP) lead to defect of cholesterol secretion into bile and subsequently led to elevation in total serum cholesterol in DZN-treated rats [36]. The increases in serum triglycerides level may be due to imbalance between synthesis rate and rate of triglycerides release by the parenchyma cells into systemic circulation [37]. With regard to HDL-c serum level, honey was able to partially recover the pesticide-induced decrease in HDL-c, whereas increased LDL-c was partially recovered with honey and combined treatment with honey DZN [30]. The increased total triglyceride could be explained by elevated adipocyte lipolysis due to DZN-induced insulin resistance and suppression of plasma hepatic lipase and lipoprotein lipase. The liver has an essential role in lipid metabolism, serving as center for lipoprotein formation, uptake and export to circulation. LDL-c and VLDL-c are main carriers of lipids from liver to peripheral cells and HDL-c transport excess cholesterol from peripheral cells to liver. Inhibited lipoprotein lipase and hepatic lipase activity together with diminished hepatic uptake due to liver destruction led to increased concentrations of serum VLDL-c and LDL-c in DZN-treated rat, respectively. The decreased HDL-c serum concentration attributed to hyperlipidemia and declined HDL-c synthesis by liver [38]. When honey was used with pesticides for treatment, the VLDL-c values showed some reduction. Many studies reported that natural agents can ameliorate hyperlipidemia that agrees with results obtained in this study [39].

Serum creatinine and urea nitrogen levels were significantly decreased in control, Honey, and Honey+ DZN groups versus DZN group. Creatinine serum level was significantly decreased in Honey group versus control, while urea nitrogen serum level was significantly decreased in Honey + DZN versus control. DZN significantly increased both creatinine and urea nitrogen levels. These increase in serum creatinine and urea nitrogen after DZN administration revealed significant impairment in renal function. Furthermore, renal problems lowered creatinine excretion, resulting in elevated blood creatinine levels. As a result, creatinine levels approximate the glomerular filtration rate. These findings are consistent with previous studies results [30,40]. Interestingly, these levels were recovered when rats were treated with honey + DZN. These data suggest that honey is good candidates for recovering pesticide-induced kidney dysfunction. The histological results of the present study revealed that rats in DZN treated group most of the kidney parenchyma showed glomerular degeneration and atrophy with leukocytic infiltration and congestion. Renal tubules showed necrotic and degenerative changes with disrupted cell borders, eosinophilic cytoplasm and congestion of blood capillaries in between degenerated tubules. In this respect, several investigations showed significant elevation of blood urea, creatinine, and uric acid levels, and renal histopathological changes in experimental animals exposed to DZN [30]. Also, Shah and Iqbal [40] revealed that rats treated daily with DZN at doses of 10, 15 and 30 mg/kg, respectively orally for 8 weeks induces kidney swelling with obliteration of space in Bowman's capsule, nuclear pyknosis, degeneration of tubular epithelial cells, necrosis of proximal tubules, flattened epithelium and congested blood vessels. It was reported that, rats treated with DZN (50 mg/kg), daily for 3 weeks showed pronounced alterations in renal corpuscle structure including a highly degeneration and necrosis of glomeruli, Bowman's capsules and associated tubules' structure. Moreover, histopathological examinations revealed that cortex is more affected than medulla. This could be partly due to uneven DZN and its metabolites distribution in renal tissue where about 90% of total renal blood flow enters cortex via bloodstream. Accordingly, a relatively high concentration of DZN and its metabolites might reach cortex via bloodstream more than medulla. In Honey treated rats; some of the glomeruli showed signs of degeneration and necrosis and appeared small while others showed normal structure. Congestion of interstitial blood capillaries was also observed, and some tubules showed signs of degeneration with unremarkable cell borders. In Honey + DZN treated rats; the kidney showed degenerative and necrotic changes in glomeruli, proximal and distal collecting tubules with pale staining cytoplasm and disappearance of the cell boundaries. Normal glomeruli were also observed in between the affected ones. Honey is a natural antioxidant, which contain flavonoids, ascorbic acid, tocopherols, catalase and phenolic compounds all of which work together to provide a synergistic antioxidant effect, scavenging and eliminating free radicals [41].

With regard to liver function, as expected and consistent with previous literature all the markers of liver injury were increased with pesticide treatment due to destruction of the membrane stability caused by increased reactive oxygen species and so release of liver enzymes from inside the cells to circulation [30,33]. Results of this study revealed that serum levels of ALT, AST, ALP and GGT were significantly increased in DZN, Honey+ DZN groups versus control; but were significantly decreased in Honey, Honey + DZN, versus DZN. Meanwhile, ALP and GGT serum levels were significantly decreased in Honey group versus control. Many studies showed that liver enzymes liberate to blood stream when hepatic parenchyma cells destructed in experimental animals exposed to DZN and other pesticides [42]. Honey administration mitigated the negative effects of pesticide on liver enzymes. These effects may contribute to antioxidant capacity of honey. In previous researches antioxidants had been proven to protect cell membrane integrity and reduce enzyme leakage by scavenging free radicals [43]. Interestingly, pesticide-related increased ALP and GGT values were returned to the normal range with honey. A possible reason for these differences could be due to the treatment duration. AST and ALT are markers of inflammation in liver which usually takes longer to resolve whereas ALP is a marker of fibrosis which doesn't occur rapidly. The results of DZN treated rats in the present study showed mild vacuolar degeneration and scattered necrosis of hepatic cells, dilatation of hepatic sinusoids, mild edema in portal area, few inflammatory cells infiltration around bile duct with mild proliferation of bile duct epithelium and congested portal vein. These results were in accordance with several research that showed elevations of hepatic enzymes and liver histopathological alterations in experimental animals exposed to DZN [30]. Also, reported that rats orally treated with DZN (50 mg/kg) daily for 3 weeks showed damage of liver structure along with disarrangement of hepatic strands, vacuolation and necrosis of hepatocytes, enlargement of hepatic sinusoids and large glycogen droplets were noted in hepatocytes cytoplasm. Also, oral administration of DZN (25 mg/kg) to rats for 15 days led to lymphocyte cells infiltration of hepatocytes [44]. It was reported that DZN (85 mg/kg) administration induced mild to moderate hepatocyte swelling (hydrophic degeneration) within 4 hours post-treatment and severe hepatocyte cell swelling and hyperemia in sinusoidal spaces within 24 hours post-treatment, and they concluded that toxic effects of DZN post-treatment increased over time period [45]. Diazinon also induced apoptosis through activating caspase 9 and 3 and increasing Bax/Bcl2 [46]. Meanwhile, the results of the present study in honey treated groups, the examination of hepatic tissue showed that they were approximately normal as control group. In honey + DZN treated rats, the hepatocytes showed mild hepatocellular vacuolar degeneration and scattered necrotic cells, some hepatocytes had pyknotic nuclei and dilated hepatic sinusoids, few and activated Kupffer cells. Honey succeeded in protecting liver against DZN toxicity by restoring the biochemical parameters and histology of liver nearly to the normal values. The improvement in liver function and structure after treatment with honey supports the antioxidant honey content (as ascorbic acid, flavonoids, catalase, phenolic compounds, and tocopherols) that had protective hepatocyte activities [47].The amelioration of oxidative stress, as a result of honey administration, was accompanied by significant reductions in size of enlarged hepatocytes and edema, restoration of bile canaliculi dilatation and reduced number of apoptotic cells [48].

In the current study tissue-deteriorating enzymes like creatine kinase and lactate dehydrogenase were measured. Consistent with previous studies, both creatine kinase and lactate dehydrogenase increased significantly with pesticide treatment. An increase in CK may represent an index of cellular necrosis and tissue damage following acute and chronic muscle injuries. The degradation and necrosis of cardiac muscle tissues causes an elevation in serum creatinine kinase in rats exposed to DZN [30,40]. Multiple studies have found that DZN and other herbicides cause cardiotoxicity in laboratory animals [49]. The present high activity of serum CK and LDH demonstrated that cellular membranes integrity of myocardial tissues disturbed. Several researchers revealed that exposure to DZN led to cardiotoxicity accompanied with elevation of CK and LDH serum levels in rats and mice [30]. Diazinon can cause apoptosis by activating caspases 9 and 3 and raising Bax/Bcl2 levels [46]. Interestingly, when treated with honey, both variables were partially normalized.

In conclusion, the findings from these experiments confirm the negative effects of DZN on body weight, biochemical measurements including blood glucose, lipid profile, kidney function tests and liver function tests and also showed degenerative effects on tissues. When rats were concomitantly treated with pesticide and honey, benefits were limited to glucose, GGT, ALP and creatine. Therefore, honey should be used as a treatment strategy to prevent pesticide-induced side effects.

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