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Research Article

Protective Effect of Arabian Balsam (*Commiphora gileadensis*) Against Hepatorenal Toxicity of Diazinon in Male Rats

Mohsen Ali Khormi[1]*, Moustafa Hussein Roshdy Elnaggar[2,3] and Mansour Attia Al-Hazmi[2]

[1]*Department of Biological Sciences, Faculty of Sciences, Jazan University, KSA*

[2]*Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, KSA*

[3]*Department of Zoology, Faculty of Sciences, Suez Canal University, Ismailia, Egypt*

***Corresponding Author:** Mohsen Ali Khormi, Department of Biological Sciences, Faculty of Sciences, Jazan University, KSA.

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Abstract

The aim of the present study was to assess the protective effect of the Arabian Balsam (*Commiphora gileadensis*) against the hepatorenal toxicity of Diazinon (DZN) on adult male albino rats. Forty male albino rats weighing 180-220 g were divided into four equal groups as follows: Group I (control group): untreated group that received 0.2 mg/kg of corn oil, daily for 6 weeks. Group II (DZN group): orally administrated 50 mg/kg of DZN in corn oil, daily for 6 weeks. Group III (Balsam group): orally administrated Arabian balsam 500 mg/kg daily for 6 weeks. Group IV (Balsam + DZN group): orally administrated 500 mg/kg balsam + 50 mg/kg DZN, daily for 6 weeks. After six weeks, blood samples were collected from retro-orbital venous plexus. Serum samples used to determine levels of liver and kidney functions. After blood sampling, livers and kidneys were isolated and fixed in 10% buffered formalin and then were processed and stained with Hematoxylin and Eosin stain and examined under light microscope. DZN administration caused significant decrease in final body weights, total proteins, albumin and high density lipoprotein cholesterol but significant increase in triglyceride, total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol, aspartate amino-transferase, alanine, aminotransferase, alkaline phosphatase, gamma glutamyl transferase, creatinine and urea nitrogen, creatinine kinase and lactate dehydrogenase enzymes compared with control and Balsam treated groups. Balsam administration led to significant decrease in blood glucose level versus control and DZN groups. In balsam + DZN group led to improvement of lipid profile, liver function tests, kidney function tests and tissue destructive enzymes. These improvements were confirmed histologically. In conclusion, DZN caused hepatorenal toxicity in rats that was improved by Arabian Balsam administration for 6 weeks. In conclusion, DZN led to hyperglycemia, dyslipidemia, alteration of liver and kidney function tests increased in tissue destructive markers as CK and LDH. Administration of balsam led to improvement of measured parameters. These improvements were confirmed histologically. In conclusion, DZN caused hepatorenal toxicity in rats that was improved by Arabian Balsam administration for 6 weeks.

Keywords: Arabian Balsam; *Commiphora gileadensis*; Diazinon; Kidney; Liver; Physiology; Histology; Rats

Introduction

Environmental pollutants such as pesticides, heavy metals, polychlorinated hydrocarbon and dioxins create serious environmental problems which affect human and animal health. Pesticides are highly poisonous organic chemical compounds that are introduced into environment to control crop pests and

disease vectors. Pesticides containing organophosphates (OP) have progressively increased in use around the world. OPs cause neurotoxicity, cardiotoxicity, hepatotoxicity, nephrotoxicity, reproductive toxicity and metabolic problems. One of extensively used OPs in agriculture is diazinon (DZN).

These effects are aggravated in case of high environmental persistence and bioaccumulation. One class of environmental pollutants consists of pesticides, which are widely used in agriculture and industry. Diazinon (DZN) is an insecticide, acaricide and nematicide with a wide spectrum contact organophosphorus (OPs) pesticide. Diazinon is extremely toxic to arthropods due to a highly specific effect on the nervous system. In mammals, however, the action of Diazinon is less well-known and may involve other mechanisms such as inflammations and tissue damage [1]. The toxicity of Diazinon results from inhibition of the acetylcholine esterase (AChE) enzyme which causes altered signal transmission due to accumulation of acetylcholine in the chemical synapses [2,3].

The Arabian balsam, *Commiphora gileadensis* (L.) (syn. *C. opobalsamum* L.) is a medium-sized aromatic shrub native to the Red Sea area, including Saudi Arabia, Yemen, Oman, Eritrea, Ethiopia, Egypt and Kenya [4]. *Commiphora gileadensis (C. gileadensis)* used historically for the therapy of many ailments and is used in traditional medicine practices in Middle East [5,6]. In Middle East, among the Arab populations, a decoction of plant aerial parts is intake as diuretic, pain reliever and laxative [7]. Iluz., *et al*. [7] investigated *C. gileadensis* medicinal properties and reported that its sap had inhibitory effect versus *Bacillus cereus* and able of blocking lectins of *Pseudomonas aeruginosa*. This indicated balsam's sap antiseptic actions. In another study, Amiel., *et al*. [8] showed that (E)-caryophyllene was a key component in *C. gileadensis* essential oil, that balsam's stem extracts acted versus cancerous tumor cells and had apoptosis-inducing effects that acted selectively, eradicating tumor cells only, but not healthy cells [9]. 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 compounds is Balsam, which acts as antioxidant.

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

Material and Methods

Materials

Both Diazinon (DZN) insecticide and Arabian Balsam (*C. gileadensis*) were purchased from the local market in and around Jeddah Provence.

Animals

Forty adult male albino rats of the Wistar strain (*Rattus norvegicus*) weighing 180–220 g were used in the 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 male albino rats were randomizing divided into 4 groups as follows: Group I: Rats of this group were untreated and served as control group and received 0.2 mg/kg of corn oil, daily for 6 weeks. Group II: Rats of this group were orally administrated 50 mg/kg of DZN in corn oil, daily for 6 weeks. Group III: Rats of this group were orally administrated 500 mg/kg b.w. of Arabian balsam daily for 6 weeks. Group IV: Rats of this group were orally administrated 500 mg/kg of Arabian balsam, then 50 mg/kg of DZN daily for 6 weeks.

Determination of LD_{50} of diazinon

The toxicity of Diazinon 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 experimental end, after 6 weeks, using a digital balance from (OHAUS, Model: Scout Pro SPU601, Made in China) [10].

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 [11]. Lipid profile was estimated in serum as triglycerides (TG) [12], total cholesterol [13], HDL-c [14]. The concentration of LDL-c was determined using [15] 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) [16], aspartate aminotransferase (AST) enzyme activity [16], alkaline phosphatase (ALP) enzyme activity [17], gamma-glutamyl transferase (GGT) [18]. Also, total bilirubin (TBIL) [19], total proteins [20] and albumin (ALB) [21]. Kidney function tests were determined as serum levels of creatinine [22] and blood urea nitrogen (BUN) [23]. Tissue destructive markers were determined as serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH) [24].

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

Weight changes in different studied groups

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

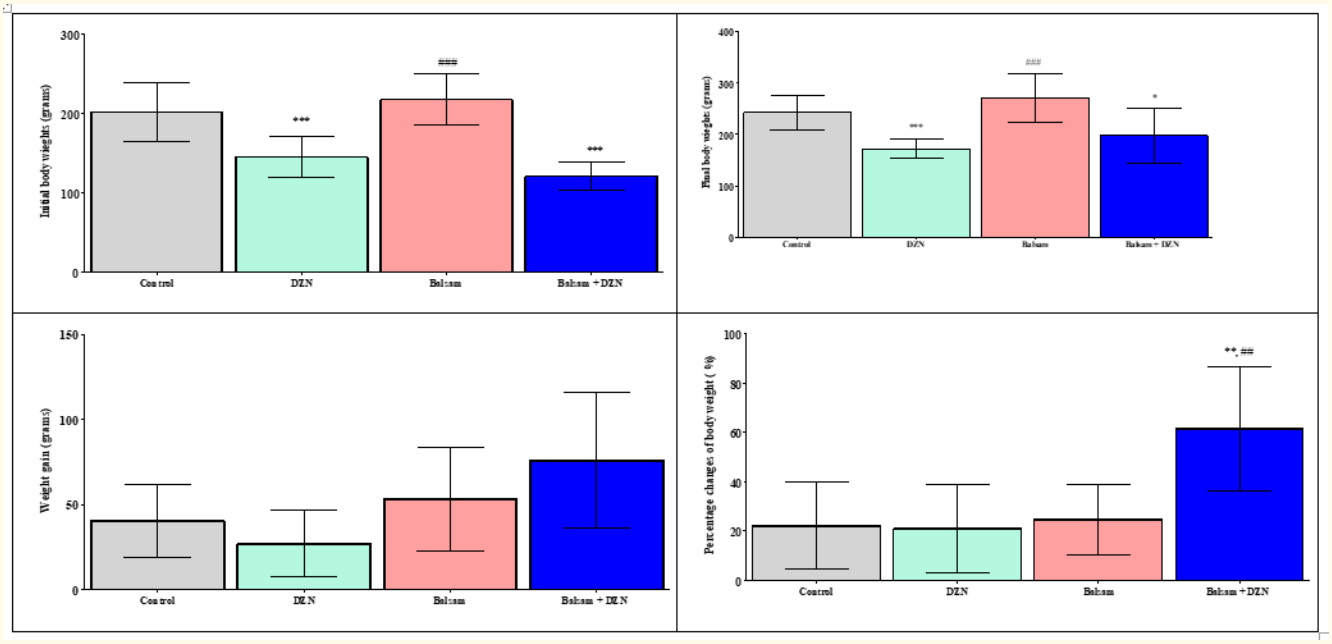


Figure 1: Initial body weights, final body weights, weight gains and percentage changes of body weights in different studied groups.

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

Levels of blood glucose, total proteins and albumin in different studied groups

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

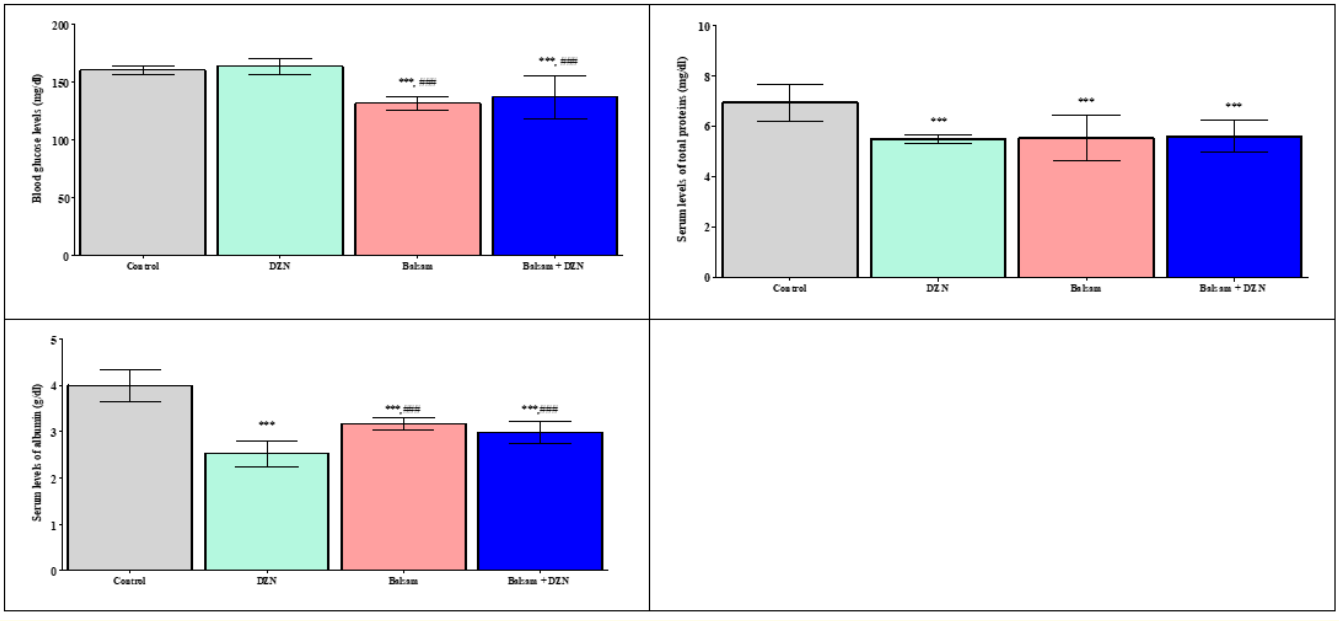
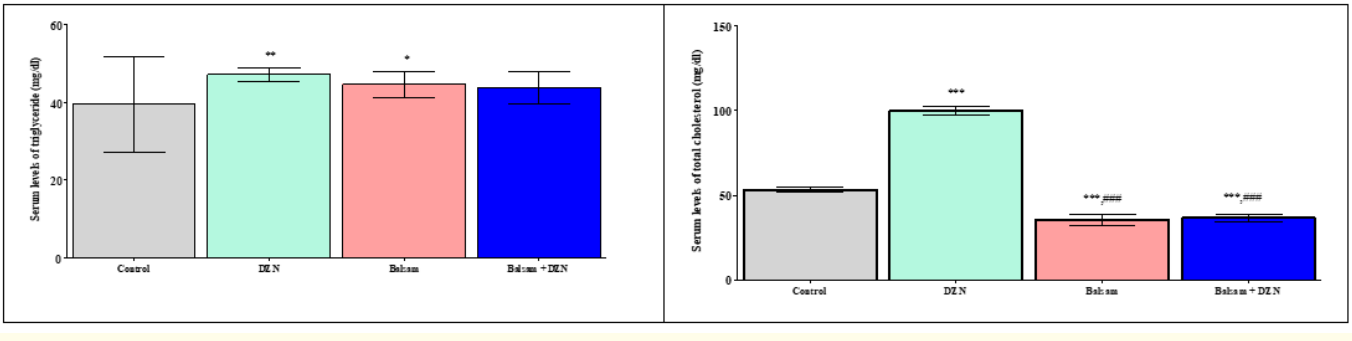


Figure 2: Levels of glucose (mg/dl), total proteins (mg/dl) and albumin (g/dl) in different studied groups. *: Significance versus control; #: Significance versus DZN group.

Levels of lipid profile in different studied groups

The serum levels of triglyceride were significantly increased in DZN, and Balsam groups versus control ($P < 0.010$, $P < 0.050$, respectively). The serum levels of total cholesterol were significantly increased in DZN versus control, Balsam and Balsam + DZN groups ($P < 0.0001$ for all) but were significantly decreased in Balsam and Balsam + DZN versus control ($P < 0.0001$ for both). The serum levels of LDL-C and vLDL-C were significantly increased in DZN versus control, Balsam and Balsam + DZN groups ($P < 0.0001$ for all). vLDL-C serum levels were significantly increased in Balsam group versus control ($P < 0.0001$). The serum levels of HDL-C were significantly decreased in DZN versus control, Balsam and Balsam + DZN groups ($P < 0.0001$ for all) but were significantly increased in Balsam and Balsam + DZN versus control ($P < 0.0001$ for both) (Figure 3).



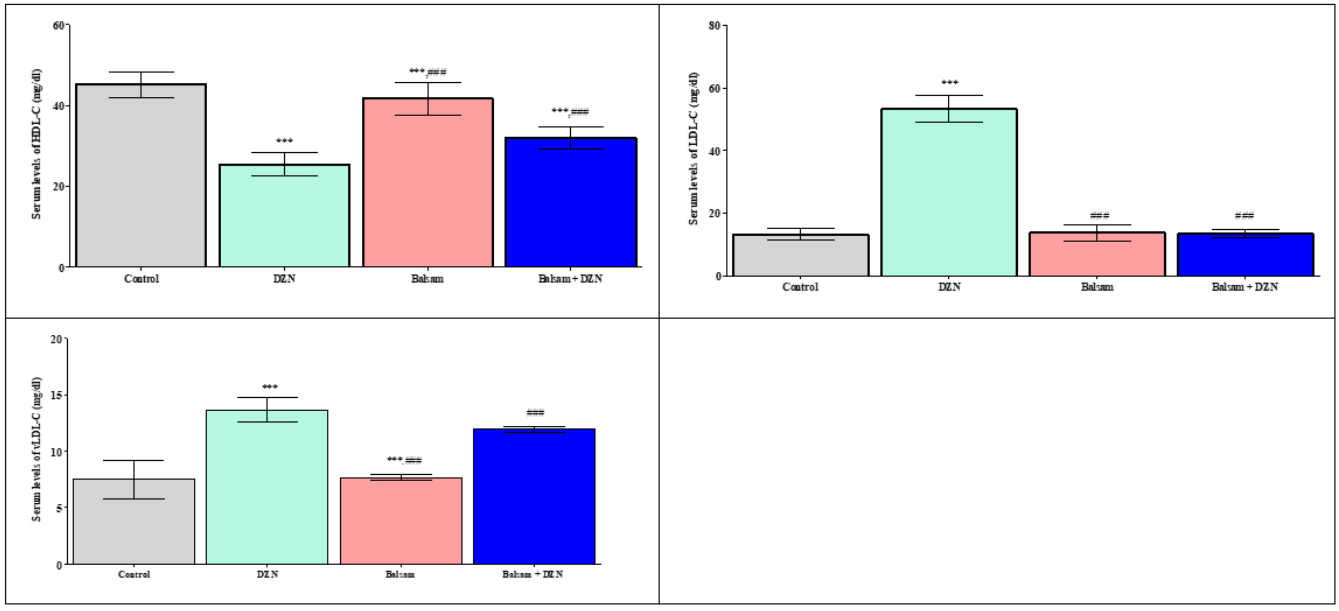


Figure 3: Levels of lipid profile in different studied groups.

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

Levels of liver enzymes activity in different studied groups

The serum levels of ALT, AST, ALP and GGT were significantly increased in DZN versus control, Balsam and Balsam + DZN groups. Serum levels of ALT and AST were significantly elevated in Balsam + DZN versus control ($P < 0.0001$). Serum levels of ALP were significantly decreased in Balsam and Balsam + DZN versus control ($P < 0.050$ for both) (Figure 4).

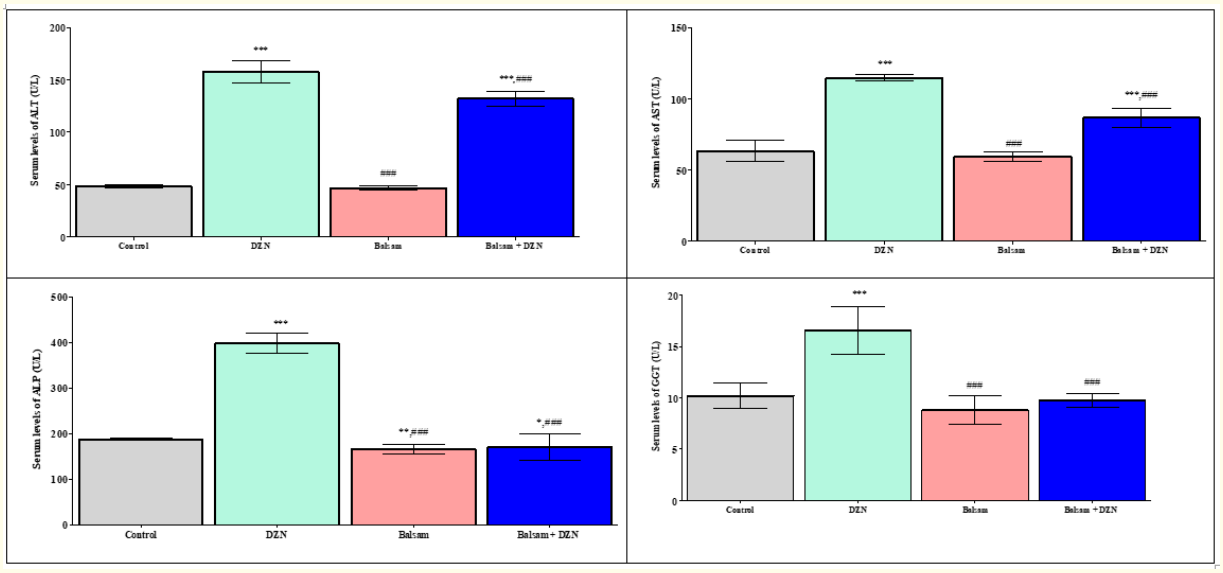


Figure 4: Liver function tests in different studied groups.

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

Levels of kidney functions tests in different studied groups

The serum levels of creatinine and urea nitrogen were significantly decreased in control, Balsam, and Balsam+ DZN groups versus DZN. Creatinine and urea nitrogen serum level were significantly decreased in Balsam group versus control ($P < 0.010$ and $P < 0.0001$) (Figure 5).

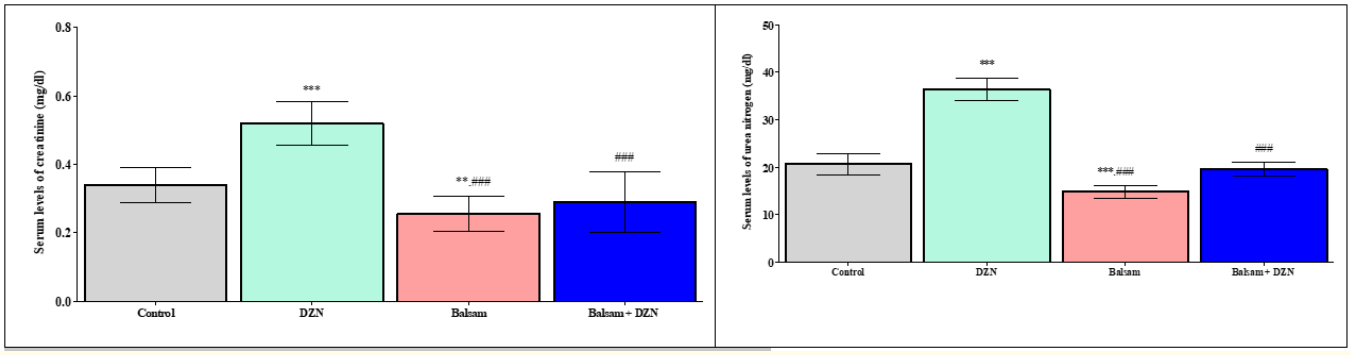


Figure 5: Kidney function tests in different studied groups.

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

Levels of tissue destructive enzymes in different studied groups:

The serum levels of CK and LDH were significantly decreased in control, Balsam, and Balsam+ DZN groups versus DZN ($P < 0.0001$) (Figure 6).

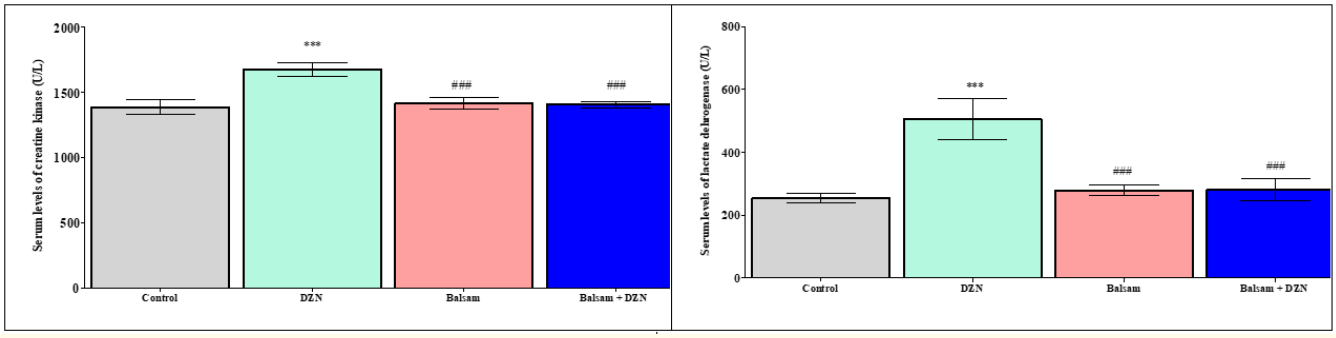


Figure 6: Serum levels of creatine kinase (U/L) and lactate dehydrogenase (U/L) in different studied groups.

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

Histological results

Histological results of liver

Liver of control rat showed normal histological structure of central vein, portal area and hepatic parenchymal cells (Figures 7A&B). 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 7C&D). Balsam treated group: Liver of rat

administrated Balsam only showed normal central veins, portal areas, and hepatic cells (Figures 7 E&F). Balsam + DZN treated rats: Liver of rats which administrated honey and toxin showed near to normal hepatic parenchymal cells, normal central vein with few degenerated cells, congested portal vessel, portal edema, and periducteolar inflammatory cells infiltration, mild hepatocellular degeneration, few necrotic cells, and few activated Kupffer cells (Figures 7 G&H).

Figure 7: A&B: Control rat group: Liver of control rat showing normal histological structure of central vein (CV), portal area (arrow) and hepatic parenchymal cells (HCs). C&D: Diazinon Treated rats: C: Portal area showed mild edema (arrow), congestion of the portal vein (CO), peri-ductal inflammatory cells infiltration (dashed arrow) with mild proliferation of bile duct epithelium and scattered hepatic cells with vacuolar degeneration (thin arrow). D: Liver parenchyma showed dilatation of the hepatic sinusoids (arrow) and mild vacuolar degeneration of the hepatic cells and scattered necrosis (dashed arrow). E&F: Balsam treated group: Liver of rat administrated Balsam only showed normal central veins (CV), portal areas (arrow), and hepatic cells (HCs). G&H: Diazinon and Balsam treated rats: G: Liver of rats which administrated honey and toxin showed congested portal vessel (CO), portal edema (arrow), and periducteolar inflammatory cells infiltration (dashed arrow). (H&E, X200). H: Liver of rat which administrated Balsam and toxin showed mild hepatocellular degeneration (short arrow), few necrotic cells (arrow), and few activated Kupffer cells (dashed arrow). H&E, A: X100; C, E and G: X200; B, D, F and 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 (Figures 8 A&B). DZN treated rat groups. Most of the kidney parenchyma showed glomerular degeneration and atrophy and leukocytic infiltration and congestion. Renal tubules were severely damaged showed necrotic and degenerative changes with disrupted cell borders and eosinophilic cytoplasm. Congestion of blood capillaries in between the degenerated tubules (Figures 8 C&D). In Balsam treated rats, the kidney architecture is normal with normal glomerulus structure. The kidney tubules appeared normal without any signs of degeneration or necrosis. The outer lines and the cell borders of the lining epithelium of the tubules are intact (Figures 8 E&F). Balsam + DZN treated rats: It showed some improvement in the renal tissue than in the Diazinon treated rats. Some of the glomeruli appeared normal in structure and in some regions; the glomeruli as well as the proximal and distal tubules showed degenerative and necrotic changes. Congestion of the interstitial blood vessels were also observed (Figures 8 G&H).

Figure 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. Renal tubules were severely damaged showed necrotic and degenerative changes with disrupted cell borders and eosinophilic cytoplasm (double arrowheads). Congestion of blood capillaries in between the degenerated tubules (B, double arrows). E&F: Balsam treated rats. The kidney architecture is normal with normal glomerulus structure (arrows) in low (A) and high Magnification (B). The kidney tubules appeared normal without any signs of degeneration or necrosis. The outer lines and the cell borders of the lining epithelium of the tubules are intact (double arrows). G&H: Diazinon and Balsam treated rats: It showed some improvement in the renal tissue than in the Diazinon treated rats. Some of the glomeruli (arrows, A) appeared normal in structure and in some regions; the glomeruli (double arrows, A) as well as the proximal and distal tubules (double arrowheads, A) showed degenerative and necrotic changes. Congestion of the interstitial blood vessels (arrowheads) were also observed. Hx&E (A, C, E&G:X100, B, D, F,&H:X400).

Discussion

The purpose of the present experimental study was to find out the protective values of Arabian Balsam (*Commiphora gileadensis*) against the toxic adverse effects of the pesticide Diazinon in adult male albino rats. The present study results revealed that there were significant decreases in initial body weight in all groups versus control group. The final body weights were significantly decreased in DZN group and Balsam + DZN group versus control. While weight gain and percentage changes in body weight in Balsam + DZN group were significantly decreased versus control but significantly increased versus DZN group. In experimental animals, OP insecticides promote body weight loss [25]. Kalender., *et al.* [26] postulated that body weight of rats decreased after 4 and 7 weeks of DZN administration. Body weights were reported to decrease significantly in rats exposed to DZN [27]. 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 direct effects of DZN [28]. Stromborg [29] 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 [23] and mice [30].

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 Balsam, Balsam+ DZN groups versus control; in Balsam, Balsam+ DZN groups versus DZN group. Insignificant changes in blood glucose observed in the present study between control and DZN group were consistent with previous studies [31]. Meanwhile, hyperglycemia was reported 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 glucose formation 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 [32]. 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 [33]. Interestingly, in the present study, when rats were treated with balsam + DZN significant decrease was observed in blood glucose levels suggesting balsam is effective in reducing blood glucose levels in rats treated with DZN. Regarding serum levels of total proteins and albumin, significant reductions were observed with DZN treated group versus control as reported previously [31,34,35]. DZN can reduce the total protein by decreasing its formation by the liver, resulting in reduction of total proteins and albumin in the blood [35]. 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 [36]. In the present study, none of the groups recovered total protein or albumin levels when administered balsam.

In the present study, serum triglycerides, total cholesterol, LDL-c and VLDL-c levels were significantly increased, while HDL-c was significantly decreased in DZN treatment group versus control. In this respect, significant increase in triglycerides and hypercholesterolemia were reported in mice treated with DZN [31]. Generally, pesticides suppressed hepatic cytochrome P-450 enzyme. Cholesterol levels increase indicates inhibitory action of pesticide on cytochrome P-450 enzyme. Moreover, 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 elevated in total serum cholesterol in DZN-treated rats [37]. Agbor., *et al*. [38] reported that, 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. In the present study, this hypercholesteremia was recovered with administration of balsam. With regard to HDL-c serum level, balsam was able to partially recover the pesticide-induced decrease in HDL-c. Whereas increased LDL-c [31] was recovered with balsam treatment. The increased 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 [39]. When balsam was used with pesticides for treatment, the VLDL-c values were reduced but not to extent of control groups (i.e., partial recovery). Many researchers reported that natural agents can ameliorate hyperlipidemia that agree with results obtained in this study [40].

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 ROS and so release of liver enzymes from inside the cells to circulation [31, 35]. Many studies showed that liver enzymes liberate to blood stream when hepatic parenchyma cells destructed in experimental animals exposed to DZN and other pesticides [41]. When individual therapy was considered, balsam treatments were not able to recover liver AST and ALT enzymes to control level. These effects may contribute to antioxidant capacity of balsam. In previous researches antioxidants have been proven to protect cell membrane integrity and reduce enzyme leakage by scavenging free radicals [42]. Interestingly, pesticide-related increased ALP and GGT values were returned to the normal range with balsam treatments. 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. Therefore, concomitant treatment with balsam may prevent the tissue damage. Whereas AST and ALT values were reduced partially with balsam treatments, values may return to normal range perhaps, if the rats are treated for longer duration.

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 [43]. Also, it was 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 [31]. Ahmadi-Naji [44] reported that oral administration of DZN (25 mg/kg) to rats for 15 days led to lymphocyte cells infiltration of hepatocytes. Hassani., *et al*. [45] 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 (ballooning degeneration) and hyperemia in sinusoidal spaces within 24 hours post-treatment. They concluded that toxic effects of DZN post-treatment increased over time period. Exposure to DZN causes hepatocytes damages [1] due to ROS production and weakened antioxidant system [46]. Diazinon also induced apoptosis through activating caspase 9 and 3 and increasing Bax/Bcl2 [47]. Meanwhile, the results of the present study in Balsam treated and honey treated groups, the examination of hepatic tissue showed that they were approximately normal as control group. In Balsam + DZN treated rats, hepatic tissues were near to normal with few hepatocytes showed degeneration and necrosis, congested portal vessel, portal edema, and periductular inflammatory cells infiltration, and few activated Kupffer cells. In the present study, in both balsam + DZN treated group, liver parenchyma showed some congested portal vessels and apparently normal hepatic cells with few degenerated cells, mild portal edema, few inflammatory cells infiltration, and proliferation of bile duct epithelium with newly formed bile ductless. In this respect, [48] reported that damage of liver tissue in alloxan induced diabetic rats caused by increase of ROS returned nearly to normal after *C. gileadensis* leaf and twig aqueous extract admiration due to *C. gileadensis* leaf and twig aqueous extract content of antioxidant compounds [7].

The 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. Also, it was 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 [49]. Al-Attar [31] 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.

Serum creatinine and urea nitrogen levels were measured to evaluate the kidney function tests and liver enzymes were measured to evaluate liver function in all studied groups. In the current study, DZN significantly increased both creatinine and urea nitrogen levels. These increases in serum creatinine and urea nitrogen 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 [31,49]. Interestingly, these levels were recovered when rats were treated with balsam + DZN. These data suggest that balsam is good candidates for recovering pesticide-induced kidney dysfunction. In Balsam treated rats, the kidney architecture, glomerulus structure and tubules were nearly normal. In Balsam + DZN treated rats the present study results showed some improvement in the renal tissue compared to DZN treated rats. Some of the glomeruli appeared normal in structure and in some regions; the glomeruli as well as the proximal and distal tubules showed degenerative and necrotic changes. Congestion of the interstitial blood vessels was also observed.

In the current study tissue-deteriorating enzymes like creatine kinase and lactate dehydrogenase were measured. Consistent with previous studies [31,49], 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. After a heart attack, creatinine kinase is the first heart enzyme to arrive in blood and it also diminishes swiftly. However, multiple studies have found that DZN and other herbicides cause cardiotoxicity in laboratory animals [43]. 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 [43,50]. Diazinon can cause apoptosis by activating caspases 9 and 3 and raising Bax/Bcl2 levels. Interestingly, when treated with balsam both variables were normalized.

Conclusion

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 Balsam, benefits were limited to tissue lipids, urea nitrogen, GGT, ALP CK and LDH. Therefore, balsam should be used as a treatment strategy to prevent pesticide-induced side effects.

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