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Protective effects of *Zygophyllum album* extract against deltamethrin-induced hyperglycemia and hepato-pancreatic disorders in rats.

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Abstract

The current study was designed to investigate the possible mechanism involved in hyperglycemia induced by chronic exposure to deltamethrin (DLM) in rat and to assess whether this damage is amenable to modulation by *Zygophyllum album*. DLM, a synthetic pyrethroid pesticide, was administrated at a dose of 4 mg/kg bw, during 60 days. Compared with control, DLM showed a significant increase of blood glucose ($p \leq 0.01$) and glycosylated hemoglobin levels ($p \leq 0.01$) and a clear decrease ($p \leq 0.01$) of insulin and total hemoglobin levels. In addition, hepatic glycogen content and the activity of hexokinase decreased ($p \leq 0.01$), whereas the activities of glucose-6-phosphatase and glycogen phosphorylase were significantly increased ($p \leq 0.01$). Moreover, pancreatic lipid peroxidation (TBARS level) was higher ($p \leq 0.01$) and oxidative stress biomarkers (SOD, CAT, GPX and GSH) were altered owing to DLM toxicity. However, *Z. album*, when combined with DLM, it significantly ameliorated almost all the hepato-pancreatic disorders induced by DLM alone. Furthermore, *Z. album* supplement were found to be effective in preserving the normal histological appearance of hepatic and pancreatic tissue. In conclusion, this study suggested that, owing to its antioxidant effects, MEZAL can potentially prevent the hyperglycemia observed in DLM-treated.

Keywords: *Zygophyllum album,* deltamethrin, hyperglycemia, pancreatic, hepatic.

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1. Introduction

The synthetic pyrethroids is a one of the most popular classes of pesticides currently used in agriculture, horticulture, forestry and public health because they are environmentally compatible by virtue of their moderate persistence, slow aqueous mobility in soil and low volatility (Erstfeld 1999). Deltamethrin (DLM) is one of the effective pyrethroid pesticide widely used extensively in the agriculture and domestic applications to control a broad spectrum of insect pests (Barlow et al. 2001). Consequently, theses pesticides can find their way into water reservoirs, streams and rivers, thus leading to adverse impacts on the aquatic organisms, animals and human health (John and Prakash 2003). Owing to its lipophilic nature, deltamethrin causes a number of toxic effects including reproductive toxicity (Benhalima et al. 2014; Chargui et al. 2009), hematotoxicity (El-Demerdash et al. 2004), hepatic dysfunction (Chargui et al. 2012), and neurotoxicity (Wolansky and Harrill 2008). Among other effects, deltamethrin has been reported to cause oxidative stress and generate reactive oxygen species (ROS) (Tuzmen et al. 2008), leading to lipid peroxidation, damage to DNA, and protein degradation (Chen et al. 2007). In addition, deltamethrin can dispose of much of the fat that enters the liver, and dysfunction in this pathway could promote the development of the nonalcoholic fatty liver disease (NAFLD). Indeed, lipids play a crucial role in the pathogenesis of diabetes mellitus (Mnafgui et al. 2015). In clinical investigation, the NAFLD is strongly correlated with insulin-resistant states such as type 2 diabetes mellitus (T2DM), and they are linked to an imbalance between apoptosis and anti apoptosis balance (Tarantino et al. 2011*b*). Moreover, several studies in experimental animal models and humans indicate a strong association between the severity of NAFLD and degree of mitochondrial dysfunction and oxidative stress (Soardo et al. 2011; Tarantino et al. 2011*a*; Videla et al. 2004). Recently, the most clinical studies have reported that long-term exposure to pesticide provokes glucose metabolism disturbance and insulin resistance (Raafat et al. 2012; Saldana et al. 2007; Slotkin

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2011). Whereas, the mechanism(s) involved in the blood glucose alterations following xenobiotics exposure have been under investigation in the recent years. Liver and pancreas are the essentially organs that control the regulation of glucose. The liver maintains the balance between the uptake and the storage of glucose via glycogenesis, and the release of glucose by the activation of processes of glycogenolysis and gluconeogenesis (Abdollahi et al. 2003). In the other hand, pancreas plays an important role in glucose homeostasis by hormonal regulation, in particular, the insulin and glucagon, and by the activation of the system transporting glucose to targeted cells. Moreover, Lasram et al. (2009) have showed that the hyperglycemia observed following malathion exposure, can be explained by a stimulation of glycogenolysis and gluconeogenesis by liver, with a temporarily loss of endocrine functions of pancreas. Furthermore, Sivakumar et al. (2009) have suggested that endogenous hepatic glucose production is increased as a result of impaired activities of the key enzymes of carbohydrate metabolism.

To prevent insulin resistance and diabetes, many synthetic drugs are available. However, most of them have been reported to cause serious side effects, when used for long time (Harrower 1994; Kiefer et al. 2004). Accordingly, a growing trend is toward the use of plant extracts, which contain a wide variety of antioxidants molecules, such as, phenolic acids, flavonoids and tannins (Corns 2003). During the past few years, halophytes have attracted the attention of researchers because they are potential sources of valuable antioxidants and have shown to exhibit various biological activities and therapeutic properties (Ksouri et al. 2008). The *Zygophyllum* species that belongs to the Zygophyllaceae family are one of the most abundant halophytes in the Mediterranean and Middle East regions. They represent a group of succulent plants that are drought resistant and/or salt tolerant, living under severe dry climatic conditions (Hammad and Qari 2010). In Tunisia, *Zygophyllum album* is one of the famous herb drug widely distributed in arid regions. This plant has many traditional uses including

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antispasmodic, antirheumatic, antieczema. It is also used as diuretic, antihistaminic and local anaesthetic agent (Moustafa et al. 2007). Research undertaken on *Z. album* showed that the dichloromethane extract of *Z. album* had appreciable *in vitro* anticancer capacity against human lung carcinoma and colon adenocarcinoma cells (Ksouri et al. 2013). In addition, recent studies in experimental animals have showed that ethanol extract of *Z. album* had a potent anti-inflammatory effect, which is manifested by decreases in CRP and TNF-α levels. Moreover, this extract had an antihyperlipidemic effect (Elgoul et al. 2012*a,b*) and could reduce the hepatotoxicity and nephrotoxicity by reverted back near to normal the values of the liver-kidney dysfunction indices (Mnafgui et al. 2012). Even more, it has been reported that oral administration of the essential oil from *Z. album* in experimental diabetic rats attenuated symptoms of diarrhea, improved lipid disorders, and hypertension through inhibiting the pancreatic lipase and angiotensin-converting enzyme (ACE) activities (Mnafgui et al. 2015).

To the best of our knowledge, the protective effects of *Z. album* have never been studied in experimental rats exposed to pesticide. Indeed, the present study was designed to investigate the antioxidant activities of MEZAL *in vitro* and to evaluate its possible protective effect against deltamethrin-induced hypergylcemia in rats. Thus, some of blood, hepatic and pancreatic key parameters of glucose homeostasis were studied. Moreover, anti- and pro-oxidant statuses, as well as histologic study of liver and pancreatic tissues, were explored too.

2. Materials and methods

2.1. Chemicals

Deltamethrin, is a synthetic pyrethroid insecticide (C22H19Br2NO3). The CAS chemical name is (a&cyano&3&phenoxybenzyl (1R,3R)&3&(2,2&dibromovinyl) & 2,2 dimethyl cyclopropanecarboxylate) and all other chemical products required for all biochemical assays was obtained from sigma Aldrich Co. (Germany).

2.2. Plant source and preparation of extract

Leaves of *Z. album* were collected from Nefta (south of Tunisian) in May 2015. The studied specie was identified by Dr. Abbes Zouhaeir, Faculty of Sciences of Gafsa, Tunisia and voucher specimens are kept at the herbarium of the Department of Life Sciences in the Faculty of Sciences of Gafsa. The Leaves were rinsed, shade dried, and reduced to fine powder. 200 g of the dried powder was extracted with 2000 ml of methanol (80%) by maceration for 48 h with frequent agitation, then the extracts were centrifuged and the supernatant was evaporated under reduced pressure to remove methanol. The dried extracts were stored at -20 °C until use.

***2.3. In vitro* antioxidant activity of MEZAL**

Hydroxyl radical scavenger ability of the methanolic extract of *Z. album* was measured using the method described by Smirnoff and Cumbes (1989). Hydroxyl radical was generated from Fenton reaction ($FeSO_4$-H_2O_2). The ability of MEZAL to scavenge hydrogen peroxide was determined according to the method describe by Ruch et al. (1989). The percentage inhibition (I%) of free radical was calculated with the following formula:

$$I\,\% = [(A_{blank} - A_{sample}) / A_{blank}] * 100$$

Radical scavenging capacity is expressed as an EC_{50} value, which is the effective concentration of the sample required to scavenge 50% of the free radicals present in the test solution. BHT was used as a reference compound.

2.4. Animals and experimental design

Adult male Wistar rats (weighing 180 - 200 g) were purchased from (SIPHAT, Tunisia). They all received a standard pellet diet purchased from the Industrial Society of Rodents' Diet

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(SNA, Sfax, Tunisia) and water *ad libitum*. All animal procedures were conducted in strict conformation with the local Institute Ethical Committee Guidelines for the Care and Use of laboratory animals of our institution. The rats were kept in metabolic cages and were housed under controlled conditions (22 - 25 °C) on a 12-h light/12-h dark cycle.

After two weeks of acclimatization, the animals were randomly divided into four different groups; six animals each. Control group, animals were given 1 ml of corn oil. *Z. album* treated group (MEZAL), animals were received daily methanolic extract of *Z. album* dissolved in corn oil (400 mg/kg bw) (Mnafgui et al. 2014); Deltamethrin treated group (DLM) animals were treated daily with deltamethrin in corn oil at a dose of 4 mg/kg bw (1/10 LD50) (Aydin 2011). Finally, animals received both deltamethrin and methanolic extract of *Z. album* (DLM+MEZAL). All the groups were treated orally (by gavage) for 60 consecutive days.

2.5. Sample collection

At the end of experimental period, the animals were fasted for 12 hours and sacrificed by cervical decapitation to avoid stress conditions. Blood samples were collected into EDTA tubes for the estimation of plasma glucose and insulin. Hemoglobin and glycosylated hemoglobin levels were estimated in whole blood samples.

The liver and pancreas tissue was dissected out, washed in ice-cold saline to remove the blood, and then a portion of the tissues was collected in 10% formalin solution and immediately processed for histological study by the paraffin technique.

Liver tissue was minced and homogenized in 0.1 M Tris– HCl buffer (pH 7.4) and centrifuged (3000 × g for 10 min). The resulting supernatant was used for the assay of key enzymes of carbohydrate metabolism. Another portion of wet liver tissue was used for the estimation of glycogen content.

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2.6. Determination of blood glucose, plasma insulin and glycosylated hemoglobin levels

Plasma glucose level was assayed by enzymatic methods based on the oxidase/peroxidase system, using commercial reagent kits, and the results were expressed as mg glucose/dl. Plasma insulin content was assayed by multi-well plate reader using the rat insulin ELISA kit, according to the manufacturer's protocol. Total hemoglobin (Hb) and glycosylated hemoglobin (HbA1c) in whole blood were spectrophotometrically analyzed by the method of Drabkin and Austin (1932). All test kits were purchased from Biomaghreb (Ariana, Tunisia).

2.7. Measurement of carbohydrate metabolic enzymes

The activities of Glucose-6-phosphatase (EC 3.1.3.9) and glycogen phosphorylase (EC 2.4.1.1) were performed as previously described by Koide and Oda (1959) and Nieymer et al. (1961), respectively, then were expressed as µmol of Pi liberated/min/mg protein. The activity of hepatic hexokinase (EC 2.7.1.1) was estimated by the method of Lapeir and Rodnick (2001) then was expressed as µmol glucose phosphorylated /min/mg of protein.

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2.8. Hepatic glycogen assay

Glycogen content was determined following the technique of Ong and Khoo (2000). The results were expressed in mg of glycogen/ g of liver.

2.9. Evaluation of pancreatic oxidative stress biomarkers

About 0.5 g of excised pancreas was homogenized into 2-ml ice-cold lyses buffer (pH 7.4), sonicated twice, and centrifuged for 20 min at 3000 × *g* and 4 °C. The collected supernatants were used for the determination of thiobarbituric acid reactive substances (TBARS) concentration, protein carbonyl levels and antioxidant enzyme activities. In this context, lipid peroxydation level was measuring by the quantification of TBARS according to Buege and

Aust (1972). In addition, protein carbonyls (PC) level was determined using the method of Levine et al. (1990). In this method, carbonyl group of proteins was measured in the resulting pellets by reaction with 2, 4-dinitrophenylhydrazine to form protein hydrazone which was measured spectrophotometrically at 370 nm.

Total superoxide dismutase (CuZn-SOD and Mn-SOD) activity was estimated spectrophotometrically according to Marklund and Marklund (1974). The absorbance was measured at 560 nm and the activity was expressed as units/mg of proteins. Catalase (CAT) activity was determined in pancreas supernatants by measuring hydrogen peroxide decomposition at 240 nm according to Aebi (1984). Glutathione-peroxidase (GPX) activity was assayed by the subsequent oxidation of NADPH at 340 nm, using the method described by Flohe and Gunzler (1984).

Total GSH and thiols contents were measured by Ellman's methods (Ellman 1959). The protein concentration was estimated by the method of Bradford (1976) using bovine serum albumin as a standard.

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2.10. Histological study

Liver and pancreas tissues were cut into small slices, and fixed in 10% buffered formalin, processed using a graded ethanol series, and then embedded in paraffin. The paraffin sections were cut into 5-µm thick slice. The pancreas was stained with hematoxylin and eosin, and the liver with periodic acid schiff (PAS) to show the glycogen deposition in hepatocytes. The sections were viewed by a light microscopic and then photographed.

2.11. Statistical Analysis

Results were expressed as mean ± standard deviation (mean ± SD). All analyses were carried out with GraphPad Prism 4.02 (GraphPad Software, San Diego,CA). Significant differences

between treatment were determined by one-way analysis of variance (ANOVA), followed by Tukey's test to correct for multiple comparisons with acceptable statistical level of significance set to 0.05.

3. Results

3.1. *In vitro* antioxidant properties

As shown in figure 1, MEZAL has a dose-dependent scavenging effect on both radical $HO^{\cdot}$ and hydrogen peroxide, compared to BHT (r^2=0.987). In the same context, EC_{50} values for $HO^{\cdot}$ and H_2O_2 scavenging activity were 335.83 ± 1.05 and 335.44 ± 1.1, respectively. Whereas the EC_{50} values of BHT were 265.65 ±1.22 and 263.28 ± 2.68µg/mL, respectively.

3.2. Acute toxicity

The dose of methanolic extract of *Z. album* (MEZAL) was non-toxic during the experiment. The lethality was found to be zero in the groups received 400 mg/kg/bw of MEZAL.

3.3. Effect of MEZAL on biochemical parameters

Table 1 depicts the levels of plasma glucose, insulin, Hb and HbA1c in control and experimental groups. Deltamethrin caused a significant ($p < 0.001$) increase in blood glucose and glycosylated hemoglobin levels as compared to the control group. Whereas, co-treatment with methanolic *Z. album* extract (DLM + MEZAL group) provoked significant decrease in these levels, as compared to deltamethrin treated group (DLM). Moreover, the levels of insulin and Hb were significantly ($p < 0.001$) decreased in the DLM-treated group. However, those levels were improved towards near normal on animals treated with MEZAL.

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3.4. Carbohydrate metabolic enzymes changes

Table 2 depicts the activities of carbohydrate metabolic enzymes hexokinase, glucose-6-phosphatase, and glycogen phosphorylase in liver tissue of control and all experimental groups. Deltamethrin treatment showed a significant ($p < 0.05$) elevation in the activities of glucose-6-phosphatase and glycogen phosphorylase, while the activity of hexokinase was significantly ($p < 0.001$) decreased. Oral co-administration of MEZAL to deltamethrin-treated animals significantly improved these parameters changes.

3.5. Effect of MEZAL on hepatic glycogen level

The treatment of rats with deltamethrin caused significant ($p < 0.001$) marked decrease in the hepatic glycogen content when compared to control group (Table 2). Simultaneous treatment of rats with deltamethrin and MEZAL caused a significant increase in the hepatic glycogen levels as compared to those treated with deltamethrin alone. However, there were no significant differences observed for the glycogen levels in rats treated with the MEZAL when compared to control group ($p \geq 0.05$).

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3.6. Lipid peroxidation and protein carbonyl changes

As shown in table 3, there was a significant increase in lipid peroxidation (TBARS) and protein carbonyls (PC) levels ($p < 0.001$) in the pancreatic organ of the deltamethrin (DLM) treated- group, as compared to control group. Further, methanolic *Z. album* extract and deltamethrin co-administration together (DLM + MEZAL group) has significantly lowered the lipid peroxidation and protein carbonyl contents in the pancreas homogenate, which remained near to that of control group.

3.7. Antioxidant enzymes and GSH pancreatic changes

The changes in the enzyme activities of the pancreatic samples of all treated groups were evaluated (Table 4). The results revealed significant decreases in superoxide dismutase (SOD), catalase (CAT) and glutathion peroxidase (GPX) activities in DLM group, as compared to control group. Interestingly, chronic treatment with MEZAL showed significant restoration in these enzymatic antioxidants activities in pancreas supernatant. Similarly, total GSH contents were lowered in deltamethrin (DLM) treated group, compared with the control group. While, a significant increase in these non- enzymatic antioxidant biomarker were observed in DLM + MEZAL group.

3.8. Histopathology examinations

H&E staining of pancreatic sections of the all treated groups are shown in Figure 2. There were no remarkable alterations in the pancreatic architecture observed in control and MEZAL treated rats (Fig. 2A, B), whereas a reduction in the size of islets, an extensive necrosis, a mild atrophy of islets and the residue of damaged β-cell population were found in the pancreas of DLM treated group (Fig. 2C). However, histological examination of the DLM + MEZAL treated rats revealed significantly reduced injuries in pancreas manifested by an increase of β-cell number and size of the islets (Fig. 2D).

Glycogen content in paraffin sections of rat liver stained with PAS method are shown in figure 3. In the liver cells of the control group, a normal content of glycogen revealed by a large number of magenta-colored fine granules distributed throughout the cytoplasm of the hepatocytes (Fig. 3A). A noticeable decrease in the deposit of glycogen was observed in the deltamethrin treated group when compared to the control (Fig. 3C). However, in deltamethrin and MEZAL treatment (Fig. 3D), we noticed an important granule of glycogen in the cytoplasm of hepatocytes when compared to deltamethrin-treated animals.

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4. Discussion

Hyperglycemia has been largely considered as one of the effects in poisoning by pesticide in human (Montgomery et al. 2008). Researchers have found that natural antioxidants derived from plant can be used for the treatment of hyperglycemia (Yolanda and Adriana 2006). *Zygophyllum album* is one of the important antidiabitic plants used in traditional medicine. However, there are no previous studies regarding the biological effects of *Z. album* against deltamethrin toxicity. Therefore, in this present study, an attempt has been made to know the possible mechanism of action through which deltamethrin induced hyperglycemia and perturbations on hepatic and pancreatic function in rats and the role of *Z. album* in ameliorating its toxic effects. The antioxidant properties *in vitro* of the MEZAL were investigated. The methanolic extract exhibited a significant hydroxyl radical scavenging activity and inhibited H_2O_2 in a dose-dependent pattern at all concentrations. Indeed, the potential scavenging ability observed in this work could be due to the presence of phenolic compounds in the leave extracts. Ksouri et al. (2013) have suggested that antioxidant activities of different extract of *Z. album* can be attributed to the presence of triterpenes, flavonoids and sterols in this plant, which are widely known as powerful antioxidants and used in various industrial fields.

The obtained antioxidant aptitude in this study can make MEZAL an excellent candidate for prevention against the *in vivo* deltamethrin-induced change on glucose homeostasis in rats. In previously experimental studies, the hyperglycemia is considered the most important complications found under organopesticides, including pyrethroids administration (Abdollahi et al. 2004). The present work revealed that chronic exposure to deltamethrin was associated with increase of blood glucose concentration and a decrease of plasma insulin levels. Similarly, Ibiang et al. (2013) and Yousef et al. (2003) described the hyperglycemia in rabbits and in rats exposed to a single intraperitoneal injection of deltamethrin. The sever hyperglycemia and hypoinsulinemia conditions in the present work might be associated to the

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alteration of pancreatic cells which was confirmed by the obtained histopathological results in this study. Many reports have suggested that the observed hyperglycemia due to insecticides toxic effects is a result of pancreatic β cell dysfunction that leading to insufficient insulin secretion (Pournourmohammadi et al. 2005).

In our study, the levels of both plasma glucose and insulin were significantly reversed after MEZAL-treatment at the concentration of 400 mg/kg for 60 days. The antihyperglycemic potential of MEZAL could be due to the increased release of insulin from the existing β cells population of the islets of langerhans and enhanced utilization of glucose by peripheral tissues. This hypothesis was further confirmed by the results of the histopathological examination that revealed an increase of β-cell number and size of the islets in DLM + MEZAL-treated rats. Indeed, the possible antihyperglycemic effects of *Z. album* can be attributed to its antioxidant properties (Elgoul et al. 2012*b*; Mnafgui et al. 2012). Previous studies have demonstrated that *Z. album* and many plants belonging to the genus *Zygophyllum* have been recommended against hyperglycemia (El Ghoul et al. 2014; Jaouhari et al. 2000). Additionally, in experimental diabetic animals, El Ghoul et al. (2012*a*) have reported that the ethanolic extract of *Z. album* at a dose of 300 mg/kg has antihyperglycemic activity with a stimulatory effect on insulin release. In accordance,, previous investigations have shown that other halophytes medicinal plants, such as *Scoparia dulcis* and *Gymnema montanum*, as well as some plant products, like morin, fraxetin and eugenol can protects the pancreatic β-cells and thereby stimulates the remnant pancreatic β-cells to synthesize and secrete more insulin (Ananthan et al. 2003; Murali et al. 2013; Pari et al. 2005;).

Recent studies showed that the induction of oxidative stress is one of the main mechanisms of deltamethrin toxicity (Tuzmen et al. 2008). It is evident that oxidative stress plays a key role in causing insulin resistance and β-cell dysfunction by their ability to activate stress-sensitive signaling pathways (Evans et al. 2003). However, to our knowledge, deltamethrin-induced

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oxidative stress in pancreas and its involvement in the ensuing hyperglycemia have not been reported. In the present study, we observed significant increase in the level of MDA and protein carbonyl content in the pancreas of deltamethrin-exposed rats which confirm the oxidative damage in pancreatic tissues. MDA and PC content are indicative of lipid peroxidation and protein oxidation in the tissues studied. Accumulating MDA level in lipid bilayer membrane of pancreas can provoke loss of its integrity and support the liberation of pancreatic enzymes into circulation (Abdollahi et al. 2004). Additionally, oxidation of protein further disrupts cellular metabolism by actively degrading key enzymes and regulating protein factors. Furthermore, glutathione, the most abundant cellular non-protein thiol, was depleted in pancreas of the rats. The depletion of GSH associated with increased MDA and PC content confirmed the occurrence of oxidative stress on the dysfunction of the endocrine function of pancreatic islets and loss of glucose control. Indeed, Kamath et al. (2007) have suggested the contribution of the oxidative stress in dimethoate-induced pancreatic damage/dysfunction leading to acute pancreatitis and hyperglycemia. Superoxide dismutase (SOD), catalase (CAT), and glutathione-peroxydase (GPx), as antioxidant enzymes**,** are first line of the defense. In this study it was clear that deltamethrin intoxication inhibited SOD, CAT and GPx activities in rat pancreas. In agreement with our results, Kamath et al. (2008) found that dimethoate induced pancreatic oxidative damage by altering SOD and CAT activities.

The co-administration of deltamethrin and MEZAL significantly decreased the oxidative stress in the pancreas. Indeed, MEZAL pretreatment significantly reduced lipid peroxidation and protein oxidation in pancreas. Furthermore, MEZAL treatment ameliorated the levels of SOD, CAT and GPx when compared to DLM-treatment. The protective effect of MEZAL could be explained by its ability to reduce the level of oxidative stress by the inhibition of ROS generation. Mnafgui et al. (2014) showed that the ethanol extract of *Z. album* helped to

protect the β-cells structure and function in diabetic rats via reduction of ROS production and ameliorated the activities of antioxidant enzymes.

Normal levels of glucose produce a normal amount of glycated hemoglobin. When the level of plasma glucose increases the fraction of glycated hemoglobin increases in a predictable way, and the total Hb level is decreased (Koeing et al. 1976). In our study, administration of MEZAL for 60 days prevented a significant elevation in HbA1c thereby increasing the level of Hb in DLM + MEAZL-treated rats. This could be due to the restoration of blood glucose level, thereby reducing the level of Hb glycosylation during the experimental period. This result is in consistent with study of Mnafgui et al. (2015), which showed that administration of essential oil of *Zygophyllum album* (OZA) significantly decreased the level of HbA1c in diabetic rats.

Maintenance of normal blood glucose levels is a particularly important function of the liver. Indeed, this organ plays a key role in glycolysis and gluconeogenesis (Pari and Srinivasan 2010). Recently, many experimental and clinical studies point out that in case of hyperglycemia or in induced diabetes, the hepatic enzyme activity of carbohydrate metabolism are markedly modulated (Sundaram et al. 2012). In this study we investigated, for the first time, the activities of hexokinase, the first enzyme of glycolysis, and glucose-6-phosphatase after 60 days of exposure to deltamethrin. Our data has demonstrated that deltamethrin decrease the hexokinase activity, whereas significant reversal in the activity of hexokinase was detected after oral administration of MEZAL to deltamethrin-treated rats. Previous studies have showed that in the absence of insulin hexokinase enzyme is almost completely inhibited in the rat liver (Gupta et al. 1999). Therefore, the elevated levels of insulin observed in DEL + MEZAL group lead to the increased hexokinase activity in the hepatic tissues there by increased glycolysis, as a result, a controlled glucose homeostasis were observed.

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On the other hand, results showed that administration of deltamethrin increased significantly the activity of glucose-6-phosphatase, enzyme implicated in mechanism of gluconeogenesis. Similar results were observed after the administration of acephate and chlorpyrifos (Acker and Nogueira 2012; Joshi and Rajini 2009). MEZAL modulated positively the activity of this enzyme leading to reducing the endogenous production of glucose.

The glycogen is considered the major storage form for carbohydrates in animals, and is the immediate source of energy. Glycogen synthase and glycogen phosphorylase are the rate-limiting enzymes in glycogen metabolism (Pederson et al. 2005). According to our result, deltamethrin impaired the normal capacity of the liver rat to synthesize glycogen, and increased the activity of glycogen phosphorylase. This finding could be due to a state of insulin deficiency, as has been reported by Parker et al. (2004). A gradual depletion of liver glycogen level in response to pesticides is also observed in albino rats (Ksheerasagar and Kaliwal 2003). The observed glycogen breakdown and increased activities of glycogen phosphorylase in deltamethrin trated-rats were normalized in DLM + MEZAL treated rats, which supported the possible role of MEZAL on the utilization and storage of glucose in the hepatic tissues.

According to Ranjbar et al. (2010), dietary supplementation with antioxidants such as polyphenols and flavonoids has beneficial effect on prevention or treatment against pesticide-induced changes in glucose homeostasis. Indeed, the potent hypoglycemic effect of MEZAL could be due to the high level of phytochemical compounds, such as kaempferol, isorhamnetin, tannins, flavoinoids, isoquercitin, β-sitosterol, and triterpenes found in the leaves of *Z. album* (Hussein et al. 2011; Moustafa et al. 2007).

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CONCLUSIONS

The current study suggests that hyperglycemia induced by deltamethrin could be mediated by induction of oxidative stress in pancreas leading to a transient dysfunction of the endocrine function of pancreatic islets and depletion of insulin secretion, as a result, a stimulation of hepatic glycogenolysis and gluconeogenesis pathways. According to its antioxidant effect, MEZAL could improve the glycemic status of deltamethrin treated-rats by stimulation of β cell function and insulin secretion and modulating the key enzymes activities of carbohydrate metabolism in hepatic tissue, such as hexokinase, glucose-6-phosphatase and glycogen phosphorylase. However, further studies are needed to gain a better understanding of the origin of oxidative stress. For this reason, it is interesting to determining the fat storage in hepatocyte of deltamethrin treated group, and accordingly adhering to the hypothesis that the exposure of hepatocytes to free fatty acids, resulting in increased ROS production and mitochondrial damage. In addition, some other parameters such as Bcl-2 protein, cytochrome c, triglycerides and unconjugated bilirubin, should be investigated.

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References

Abdollahi, M., Chan, T.S., Subrahmanyam, V., and O'Brien, P.J. 2003. Effects of phosphodiesterase 3,4,5 inhibitors on hepatocyte cAMP levels, glycogenolysis, gluconeogenesis and susceptibility to a mitochondrial toxin. Mol. Cell. Biochem. **252**: 205–211.

Abdollahi, M., Donyavi, M., Pournourmohammadi, S., and Saadat, M. 2004. Hyperglycemia associated with increased hepatic glycogen phosphorylase and phosphoenolpyruvate carboxykinase in rats following subchronic exposure to malathion. Comp. Biochem. Physiol. C. **137**: 343–347.

Acker, C.I., and Nogueira, C.W. 2012. Chlorpyrifos acute exposure induces hyperglycemia and hyperlipidemia in rats. Chemosphere, **89**: 602–608.

Aebi, H. 1984. Catalase *in vitro*. Methods Enzymol. **105**: 121 – 126.

Ananthan, R., Baskar, C., NarmathaBai, V., Pari, L., Latha, M., el al. 2003. Antidiabetic effect of Gymnemamontanum leaves: effect on lipid peroxidation induced oxidative stress in experimental diabetes. Pharmacol. Res. **48**: 551–556.

Aydin, B. 2011. Effects of thiacloprid, deltamethrin and their combination on oxidative stress in lymphoid organs, polymorphonuclear leukocytes and plasma of rats. Pest. Biochem. Physiol. **100**: 165-171.

Barlow, S.M., Sullivan, F.M., and Lines, J. 2001. Risk assessment of the use of deltamethrin on bednets for the prevention of malaria. Food Chem. Toxicol. **39**: 407–422.

Benhalima, N., Benslima, A., Moalla, I., Fetoui, H., Pichon, C., et al. 2014. Protective effects of oat oil on deltamethrin-induced reprotoxicity in male mice. Food Funct. **5**: 2070-2077.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgramquantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**: 248.

Buege, J.A., and Aust, S.D. 1972. Microsomal lipid peroxidation. Methods Enzymol. **51**: 302–310.

Chargui, I., Grissa, I., BenSassi, F., Hrira, M.Y., Haouem, S., et al. 2012. Oxidative stress, biochemical and histopathological alterations in the liver and kidney of female rats exposed to low doses of deltamethrin (DM): A molecular assessment. Biomed. Environ. Sci. **25**: 672-683.

Chargui, I., Haouem, S., Haouas, Z., Zaouali, M., and BenCheikh, H. 2009. Toxic responses to deltamethrin (DM) low doses on gonads, sex hormones and lipoperoxidation in male rats following subcutaneous treatments. J. Toxicol. Sci. **34**: 663-670.

Chen, D., Huang, X., Liu, L., and Shi, N. 2007. Deltamethrin induces mitochondrial membrane permeability and altered expression of cytochrome C in rat brain complications of sulfonylureas. J. Diabetes Complication, **8**: 201– 203.

Draft

Corns, C.M. 2003. Herbal remedies and clinical biochemistry. Ann. Clin. Biochem. **40**:489–507.

Drabkin, D.L., and Austin, H.J. 1932. Spectrophotometric studies. I. Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. J. Biol. Chem. **98**: 719 –733.

El-Demerdash, F.M., Yousef, M.I., Kedwany, F.S., and Baghdadi, H.H. 2004. Role of alpha-tocopherol and beta-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hemato biochemical parameters, and semen quality of male rats. J. Environ. Sci. Health B. **39**: 443–459.

El Ghoul, J., Boughattas, N.A., and Ben-Attia, M. 2012*a*. Antihyperglycemic and antihyperlipidemic activities of ethanolic extract of *Zygophyllum album* in streptozotocin-induced diabetic mice. Toxico. Indust. Health, **29**: 43–51

El Ghoul, J., Smiri, M., Ghrab, S., Boughattas,N.A., and Ben-Attia, M. 2012*b*. Antihyperglycemic, antihyperlipidemic and antioxidant activities of traditional aqueous extract of *Zygophyllum album* in streptozotocin diabetic mice. Pathophysiology, **19**: 35–42.

El Ghoul, J., and Ben-Attia, M., 2014. Vasorelaxant effects of aqueous extract of *Zygophyllum Album* and antihyperglycemic activities in streptozotocin-induced diabetic mice. J. Diabetes Metab. **5**: 426.

Ellman , G.L. 1959. Tissue sulfhydryl groups, Arch. Biochem. Biophys. **82**: 70 – 77.

Erstfeld, K.M. 1999. Environmental fate of synthetic pyrethroids during spray drift and field runoff treatments in aquatic microcosms. Chemosphere, **39**: 1737–1969.

Evans, J.L., Goldfine, I.D., Maddux, B.A., and Grodsky, G.M. 2003. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction. Diabetes, **52**: 1–8.

Flohe, L., and Gunzler, W.A. 1984. Assays of glutathione peroxidase. Methods Enzymol. **105**: 114 – 121.

Gupta, D., Raju, J., Prakash, J.R., and Baquer, N.Z. 1999. Change in the lipid profile, lipogenic and related enzymes in the livers of experimental diabetic rats, effect of insulin and vanadate. Diabetes Res. Clin. Pract. **46**: 1–7.

Hammad, I., and Qari, S.H. 2010. Genetic diversity among *Zygophyllum* (Zygophyllaceae) populations based on RAPD analysis. Genet. Mol. Res. **9**: 2412-2420.

Harrower, A.D. 1994. Comparison of efficacy, secondary failure rate, and complications of sulfonylureas. J. Diabetes Complication, **8**: 201– 203.

Hussein, S.R., Marzouk, M.M., Ibrahim, L.F., Kawashty, S.A., and Saleh, N.A.M. 2011. Flavonoids of *Zygophyllum album L.f.* and *Zygophyllum simplex L., (Zygophyllaceae*). Biochem. Syst. Ecol. **39**: 778-780.

Draft

Ibiang, Y.B., Ekaluo, U.B., Nta, A.I., Ikpeme, E.V., Ekanem, B.E., et al. 2013. Effect of deltamethrin and ridomil on serum biochemical parameters in the rat :Rattus Norvegius. Eur. J. Toxicol. Sci. **5**: 2052-5230.

Jaouhari, J.T., Lazrek, H.B., and Jana, M. 2000. The hypoglycemic activity of Zygophyllum gaetulum extracts in alloxan induced hyperglycemic rats. J. Ethnopharmacol. **69**: 17–20.

John, P.J., and Prakash, A., 2003. Bioaccumulation of pesticides on some organs of fresh water catfish Mystis vitatus. Bull. Environ. Contam. Toxicol. **70**: 1013–1016.

Joshi, A.K., and Rajini, P.S. 2009. Reversible hyperglycemia in rats following acute exposure to acephate, an organophosphorus insecticide: role of gluconeogenesis. Toxicology, **257**: 40–45.

Kamath,V., and Rajini P.S. 2007. Altered glucose homeostasis and oxidative impairment in pancreas of rats subjected to dimethoate intoxication. Toxicology, **231**: 137-146.

Kamath, V., Joshi A.K.R., and Rajini P.S. 2008. Dimethoate induced biochemical perturbations in rat pancreas and its attenuation by cashew nut skin extract. Pestic. Biochem. Physiol. **90**: 58-65.

Kiefer, F., and Wiedmann, K. 2004. Combined therapy: what does acamprosate and naltrexone combination tell us? Alcohol Alcohol, **39**: 542–547.

Koeing, R.J., Peterson, C.M., Jone, R.L., Saudek, C., Lehman, M., et al. 1976. Correlation of glucose regulation and haemoglobin A1c in diabetes mellitus. N. Engl. J. Med. **295**: 417- 420.

Koida, H., and Oda, T. 1959. Pathological occurrence of glucose-6-phosphatase in liver. diseases. Clin. Chem. Acta, **4**: 554-561.

Ksheerasagar, R.L., and Kaliwal, B.B. 2003. Temporal effects of mancozeb on testes, accessory reproductive organs and biochemical constituents in albino mice. Environ. Toxicol. Phar. **15**: 9–17.

Ksouri, R., Megdiche, W., Falleh, H., Trabelsi, N., Boulaaba, M., et al. 2008. Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. C. R. Biologies, **331**: 865–873.

Ksouri,W.M., Mdini,F., Mkadmi,K., Legaut, J., and Magné, C. 2013. LC–ESI-TOF–MS identification of bioactive secondary metabolites involved in the antioxidant, anti-inflammatory and anticancer activities of the edible halophyte *Zygophyllum album* Desf. Food Chem. **139**: 1073–1080.

Lapeir, T.K., and Rodnick, K.J. 2001. Effects of aerobic exercise on energy metabolism in the hypertensive rat heart. Phys. Ther. **81**: 1006-1017.

Lasram, M.M., Annabi, A.B., Rezg, R., Elj, N., Slimen, S., et al. 2009. Metabolic disorders of acute exposure to malathion in adult Wistar rats. J. Hazard. Mater. **163**: 1052–1055.

Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., et al. 1990. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. **186**: 464– 478.

Draft

Marklund, S., and Marklund,G. 1974. Involvement of the super-oxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. **47**: 469–474.

Mnafgui, K., Hamden, K., Ben Salah, H., Kchaou, M., Nasri, M., et al. 2012. Inhibitory Activities of *Zygophyllum album*: A Natural Weight-Lowering Plant on Key Enzymes in High-Fat Diet-Fed Rats. Evid. Based Complement. Alternat. Med. **2012**: ID 620384, 9 p.

Mnafgui, K., Kchaou, M., Hamden, K., Derbali, F., Slama, S., et al. 2014. Inhibition of carbohydrate and lipid digestive enzymes activities by *Zygophyllum album* extracts: effect on blood and pancreas inflammatory biomarkers in alloxan-induced diabetic rats. J. Physiol. Biochem. **70**: 93-106.

Mnafgui, K., Kchaou, M., Ben Salah, H., Hajji, R., Khabbabi, G., et al. 2015. Essential oil of *Zygophyllum album* inhibits key-digestive enzymes related to diabetes and hypertension and attenuates symptoms of diarrhea in alloxan-induced diabetic rats. Pharm. Biol. **6**: 1-8.

Montgomery, M.P., Kamel, F., Saldana, T.M., Alavanja, M.C., and Sandler, D.P., 2008. Incident diabetes and pesticide exposure among licensed pesticide applicators: Agricultural Health Study, 1993–2003. Am. J. Epidemiol. **167**: 1235–1246.

Moustafa, A.M.Y., Khodhair, A.I., Hammouda, F.M., and Husseiny, H.A. 2007. Phytochemical and toxicological studies of *Zygophyllum album* L.F. J. Pharma. Toxicology, **2**: 220-237.

Murali, R., Srinivasan, S., and Ashokkumar, N. 2013. Antihyperglycemic effect of fraxetin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-induced diabetic rats. Biochimie, **95**: 1848-1854.

Nieymer, H., Gonzalez, C., and Rozzi, R. 1961. Influence of diet on liver phosphorylase: effects of fasting and refeeding. J. Biol. Chem. 236:610-613.

Ong, K.C., and Khoo, H.E. 2000. Effects of myricetin on glycemia and glycogen metabolism in diabetic rats. Life Sci. **67**: 1695-1705.

Pari, L., and Latha, M. 2005. Antidiabetic effect of Scopariadulcis: effect on lipid peroxidation in streptozotocin diabetes. Gen. Physiol. Biophys. **24**: 13–26.

Pari, L., and Srinivasan, S. 2010. Antihyperglycemic effect of diosmin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-nicotinamide-induced diabetic rats. Biomed. Pharmacotherapy, **64**: 477-481.

Parker, G., Taylor, R., Jones, D., Mc. and Clain, D. 2004. Hyperglycemia and inhibition of glycogen synthase in streptozotocin-treated mice: role of O-linked N acetyl gluco-samine. J. Biol. Chem. **279**: 20636 –20642.

Pederson, B.A., Schroeder, J.M., Parker, G.E., Smith, M.W., DePaoli-Roach, A.A., et al. 2005. Glucose metabolism in mice lacking muscle glycogen synthase. Diabetes, **54**: 3466-3473.

Draft

Pournourmohammadi, S., Farzami, B., Ostad, S.N., Azizi, E., and Abdollahi, M., 2005. Effects of malathionsubchronic exposure on rat skeletal muscle glucose metabolism. Environ. Toxicol. Pharmacol. **19**: 169–191.

Raafat, N., Abass, M.A., and Salem, H.M. 2012. Malathion exposure and insulin resistance among a group of farmers in Al-Sharkia governorate. Clin. Biochem. **45**: 1591–1595.

Ranjbar, A., Ghahremani, M.H., Sharifzadeh, M., Golestani, A., Ghazi-Khansari, M., et al. 2010. Protection by pentoxifylline of malathion-induced toxic stress and mitochondrial damage in rat brain. Hum. Exp. Toxicol. **29**: 851–864.

Ruch , R.J. , Cheng ,S.J., and Klaunji, J.E. 1989. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, **10**: 1003–1008.

Saldana, T.M., Basso, O., Hoppin, J.A., Baird, D.D., Knott, C., et al. 2007. Pesticide exposure and self-reported gestational diabetes mellitus in the Agricultural Health Study. Diabetes Care, **30**: 529–534.

Sivakumar, S., and Subramanian, S.P. 2009. Pancreatic tissue protective nature of D-Pinitol studied in streptozotocin-mediated oxidative stress in experimental diabetic rats. E. J. Pharm. **622**: 65 –70.

Slotkin, T.A. 2011. Does early-life exposure to organophosphate insecticides lead to prediabetes and obesity? Reprod. Toxicol. **31**: 297–301.

Smirnoff, N., and Cumbes, Q.J. 1989. Hydroxyl radical scavenging activity of compatible Solutes. Phytochemistry, **28**: 1057–1060.

Soardo, G., Donnini, D., Domenis, L., Catena, C., De Silvestri, D., et al. 2011. Oxidative stress is activated by free fatty acids in cultured human hepatocytes._Metab. Syndr. Relat. Disord. **9**: 397-401

Sundaram, R., Naresh, R., Shanthi, P., and Sachdanandam, P. 2012. Efficacy of 20-OH-ecdysone on hepatic key enzymes of carbohydrate metabolism in streptozotocin induced diabetic rats. Phytomedicine, **19**: 725-729.

Tarantino, G., Scopacasa, F., Colao, A., Capone, D., Tarantino, M., et al. 2011*a*. Serum Bcl-2 concentrations in overweight-obese subjects with nonalcoholic fatty liver disease. World J. Gastroenterol. **17**: 5280-5288.

Tarantino,G., Colao,A., Capone,D., Tarantino, M., Conca P., et al. 2011*b*. Circulating levels of cytochrome C, gamma-glutamyl transferase, triglycerides and unconjugated bilirubin in overweight/obese patients with non-alcoholic fatty liver disease. J. Biol. Regul. Homeost. Agents, 25:47-56.

Tuzmen, N., Candan, N., Kaya, E., and Demiryas N. 2008. Biochemical effects of chlorpyrifos and deltamethrin on altered antioxidant defense mechanisms and lipid peroxidation in rat liver. Cell. Biochem. Funct. **26**: 119-124.

Draft

Videla, L.A., Rodrigo, R., Orellana, M., Fernandez, V., Tappia, G., et al. 2004. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. Clinical Science Mar. **106**: 261-268

Wolansky, M.J., and Harrill, J.A. 2008. Neurobehavioral toxicology of pyrethroid insecticides in adult animals: a critical review. Neurotoxicol. Teratol. **30**: 55– 78.

Yolanda, B.L, and Adriana, G.C. 2006. Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. J. Nutr. Biochem. **17**: 1–13.

Yousef, M.I., El-Demerdash, F.M., Kamel, K.I., and Al-Salhen, K.S. 2003. Changes in some hematological and biochemical indices of rabbits induced by isoflavones and cypermethrin. Toxicology, **189**: 223–234.

Draft

Table 1

Effect of methanolic extract of *zygophyllum album* leaves (MEZAL) on changes in blood glucose level, plasma insulin level, total hemoglobin (Hb) and glycosylated hemoglobin (HbA1c) in control and experimental rats.

Parameters and Treatments	Control	MEZAL	DLM	DLM + MEZAL
Glucose (mg/dl)	122.3 ± 1.65	123.3 ± 2.42	131.7 ± 1.81 ***	124.4 ± 3.03 ¥¥¥
Insulin (µU/ml)	17.03 ± 1.55	15.63 ± 0.49	9.2 ± 0.55 ***	13.13 ± 0.73 ¥¥
Total hemoglobin (Hb) (g/dl)	16.49 ± 0.94	15.99 ± 0.89	12.17 ± 1.31 ***	14.19 ± 1.08 ¥
Glycated hemoglobin (HbA1c) (mg/g Hb)	0.36 ± 0.03	0.36 ± 0.07	1.01 ± 0.07 ***	0.52 ± 0.04 ¥¥¥

Values are presented as a mean ± SD (n = 6). * $p < 0.05$, ** $p < 0.01$, *** $P<0.001$ vs control; and ¥ $P<0.05$, ¥¥ $P<0.01$, ¥¥¥ $P<0.001$ vs DLM

Draft

Table 2

Effect of methanolic extract of *zygophyllum album* leaves (MEZAL) treatment on glycogen content and hepatic activities of Hexokinase, Glucose-6-phosphatase and Glycogen phosphorylase in control and experimental rats.

Parameters and Treatments	Control	MEZAL	DLM	DLM + MEZAL
Hexokinase (µmol glucose phosphorylated /min/mg of protein)	21.72 ± 1.23	20.43 ± 1.42	10.37 ± 0.6***	15.47 ± 0.68 ¥¥
Glucose-6-phosphatase (µmol of Pi liberated/min/mg protein)	20.57 ± 1.24	18.82 ± 1.6	23.83 ± 2.55*	19.97 ± 1.81 ¥¥
Glycogen phosphorylase (µmol of Pi liberated/min/mg protein)	10.92 ± 1.07	11.30 ± 1.13	12.78 ± 0.84 *	12.99 ± 1.18 ¥
Glycogen (mg /g of liver)	11.47 ± 1.21	11.04 ± 1.05	7.18 ± 1.08 ***	9.28 ± 1.14 ¥

Values are presented as a mean ± SD (n = 6). * $p < 0.05$, ** $p < 0.01$, *** $P<0.001$ vs control; and ¥ $P<0.05$, ¥¥ $P<0.01$, ¥¥¥ $P<0.001$ vs DLM

Draft

Table 3

Effects of methanolic extract of *zygophyllum album* leaves (MEZAL) on lipid peroxidation (TBARS) and protein carbonyls (PC) contents in the pancreatic tissue of control (C) and experimental rats.

Parameters and treatments	**C**	**MEZA**	**DLM**	**DLM + MEZA**
TBARS (nmoles MDA/ mg protein**)**	9.48± 0.51	9.1± 0.48	15.05± 1.03***	12.9±1.05¥¥¥
PC (nmoles/mg protein)	4.27± 0.17	4.1± 0.59	6.73± 0.44***	5.72± 0.59¥¥

Values are presented as a mean ± SD (n = 6). * p < 0.05, ** p < 0.01, *** P<0. 001 vs control; and ¥ P <0. 05, ¥¥ P <0.01, ¥¥¥ P < 0. 001 vs DLM

Draft

Table 4

Effects of methanolic extract of *zygophyllum album* leaves (MEZAL) extract on enzymatic and non-enzymatic antioxidants changes in the pancreatic tissue of control (C) and experimental rats.

Parameters and treatments	C	MEZA	DLM	DLM + MEZA
GSH (µmole / g tissue)	207.9± 3.05	203.9± 2.74	192.4± 1.98***	196.6 ±1.39 ¥
SOD (U/mg protein)	15.05±0.59	15.47±0.78	13.87±0.79*	15.11±0.65 ¥
CAT(µmol of H2O2 destroyed/min/mg protein)	14.23±0.75	14.97±0.71	11.95±1.03***	13.63±0.37 ¥¥
GPx(nmol of NADPH oxidized/min/mg protein)	8.97±0.52	9.68±0.95	7.22±0.58**	8.56±0.75 ¥

Values are presented as a mean ± SD (n = 6). * p < 0.05, ** p < 0.01, *** P<0. 001 vs control; and ¥ P <0. 05, ¥¥ P <0.01, ¥¥¥ P < 0. 001 vs DLM

Draft

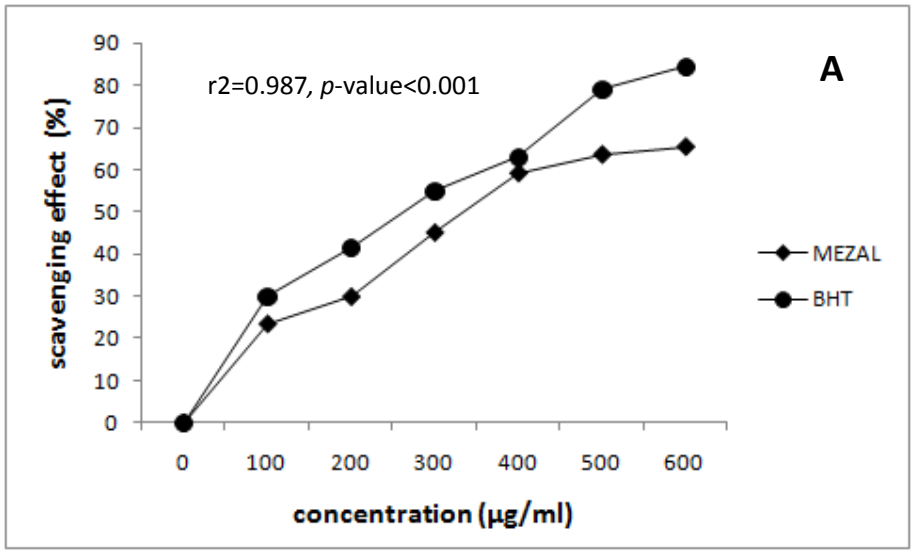
Figure captions

Figure 1. Free radical scavenging activities of methanol extract of *Z.album* leaves. (A) hydroxyl radical scavenging activities of various concentrations of MEZAL (100–600 µg/ml) and BHT. (B) hydrogen peroxide scavenging activities of MEZAL (100–600 µg/ml) and BHT.

Figure2. Photomicrographs of pancreatic tissues in control and experimental treated rats. Pancreatic tissue sections stained with hematoxylin-eosin (G x 400). (A) in control rats, representative micrograph shows a normal pancreatic islet (→), (B) treated with MEZAL (400 mg/kg) alone showing normal cluster islet cells (→); (C) in deltamethrin treated rats: showing a significant reduction in the size of islets, damaged β -cell population and extensive necrotic changes also (⇝), (D) co-treated with deltamethrin and MEZAL, representative micrograph show the increase of islets size and the disappearance of necrotic changes, showing apparently normal architecture (→).

Draft

Figure 3. Photomicrograph of the liver tissues in control and experimental treated rats (sections stained with Periodic acid-Schiff (PAS) for detection of glycogen deposition). (A) in control rats and (B) in MEZAL treated groups, representative micrographs showing a positive PAS- reaction, with magenta color staining and a homogenously glycogen distribution in the hepatocytes (→), (C) in deltamethrin treated rat showing a significant apparent decrease of glycogen deposition in the hepatocytes (⇝). (D) co-treated with deltamethrin and MEZAL, representative micrographs shown a remarkable increase in glycogen deposition in hepatocytes close to that of control rats (◯). Original magnification (G x 400).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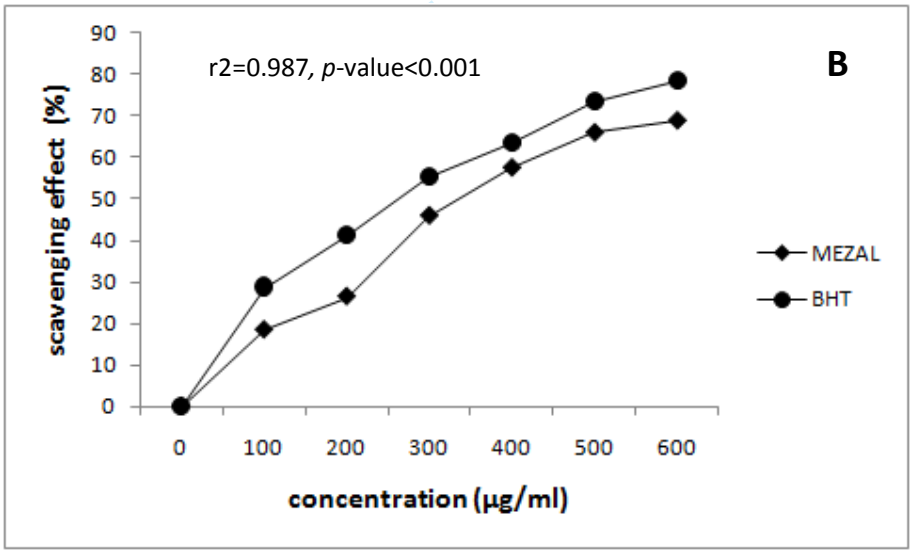
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Figure 1

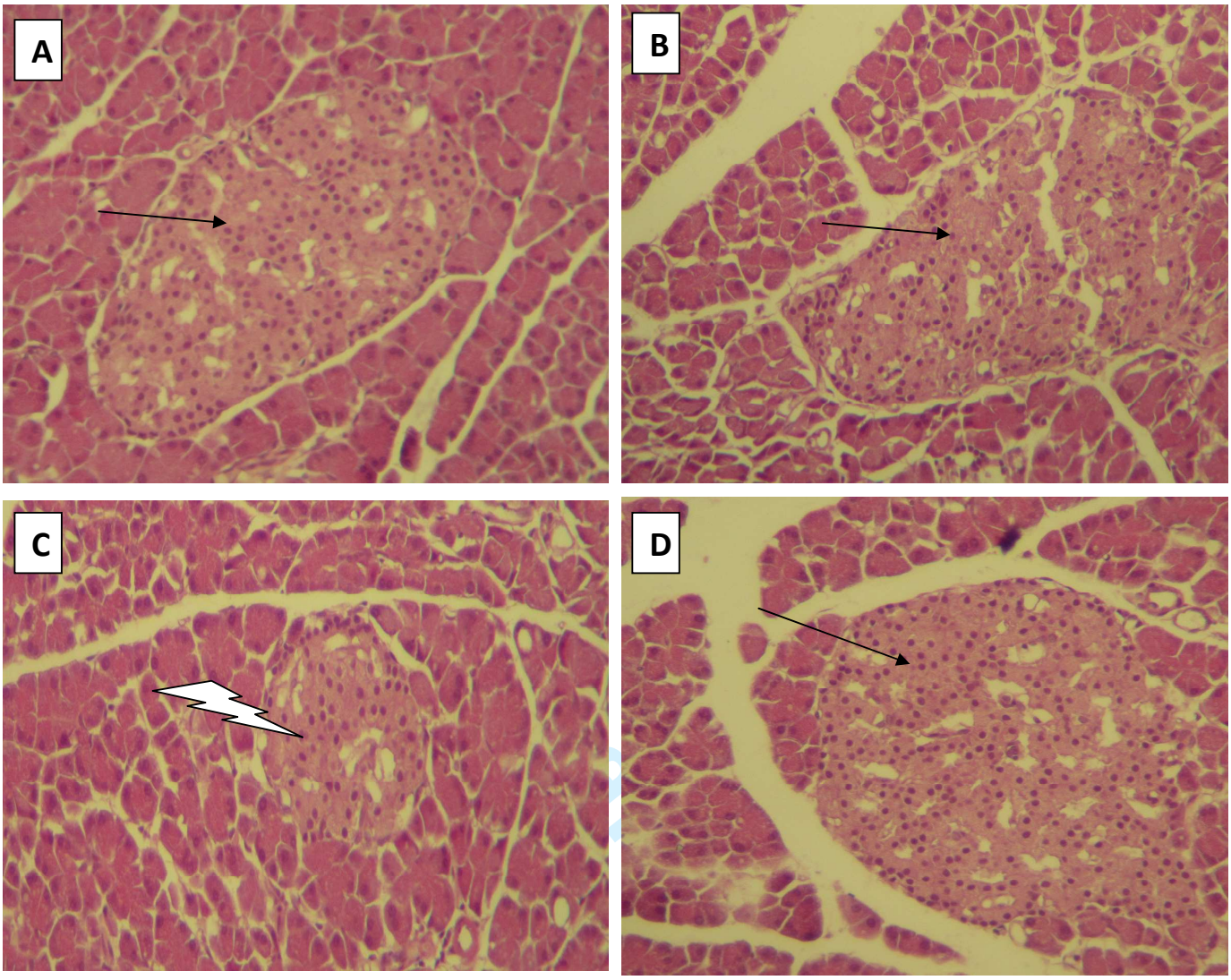

Figure 2

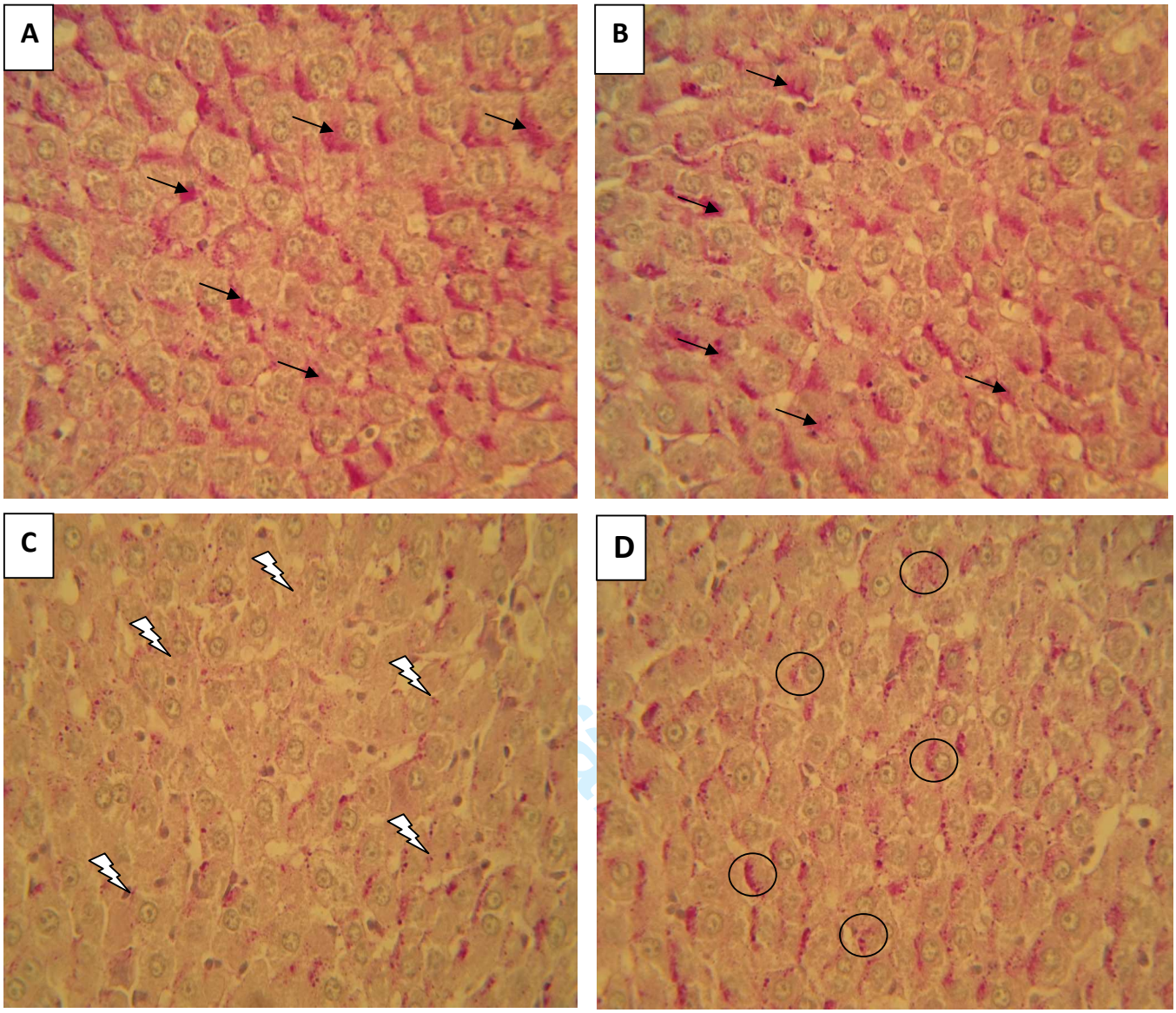

Figure 3