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Optimizing the therapeutic dose of *Leiurus quinquestratus* scorpion venom in type-2 diabetic mellitus rats

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ABSTRACT

Diabetes mellitus (DM) is a common chronic disease. Recently, *Leiurus quinquestratus* venom (LQV) showed anti-diabetic effect against type-2 DM (T2-DM). This study aimed to optimize the therapeutic dose LQV in T2-DM rats. Forty-eight male rats were divided into eight groups (n=6) as follows; group 1 (Gp1) was served as a control. From Gp2 to Gp8, rats were fed in high fat diets (HFD), and then injected with a single dose of streptozotocin (30 mg/kg b.wt). Then, Gp2 was left as untreated T2-DM, while groups from Gp3 to Gp8 were treated with metformin (Met) (150 mg/kg b.wt), or with different doses of LQV (1/10 to 1/320 LD_{50}). Post a month of daily treatments; the body weight changes, biochemical parameters, and liver histopathological changes were recorded. The results showed that the glucose level had increased and accompanied with sever alterations in the liver architectures in T2-DM rats (Gp2). The treatment of T2-DM rats with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} decreased the glucose level, ameliorated the biochemical changes, and improved the liver architectures. However, the treatment of T2-DM rats with 1/160 or 1/320 LQV LD_{50}, did not show significant changes when compared to T2-DM rats. Collectively, the therapeutic dose of LQV that could be used to treat T2-DM rats ranged between 1/10 to 1/80 of LQV LD_{50}

Keywords:
Antioxidant enzymes, Glucose, *Leiurus quinquestratus* venom, T-2 diabetes mellitus.

1. Introduction

Diabetes mellitus (DM) is a complex endocrine and metabolic disorder that can lead to complications that cause end-organ damage. It represents a serious public health problem worldwide **(**Sarkar *et al*., 2019**).** DM is a chronic disorder of carbohydrates, fats, and protein metabolism. A defective or deficient insulin secretary response, which translates into impaired glucose use, is a characteristic feature of diabetes mellitus, as is the resulting hyperglycemias **(**Papatheodorou *et al*., 2018**).** Impaired glucose metabolism led to chronic inflammation, hyper-insulinemia, insulin resistance, or oxidative stress, cardiovascular disease **(***Pushpani* et al., 2016). DM is the most common endocrine disorder and usually occurs when there is deficiency or absence of insulin or rarely, and impairment of insulin activity (insulin resistance) **(**Karalliedde et al., 2016**).**

There were several sub-classifications of DM, including type 1(T1-DM), type 2 (T2-DM), gestational diabetes, neonatal diabetes, and steroid-induced diabetes. T1-DM and T2-DM are the main subtypes, each with different pathophysiology and management, but both have a potential for hyperglycemia **(**Singh et al**.,** 2016**)**.

T2-DM is caused by a combination of two primary factors: defective insulin secretion by

pancreatic β-cells and the inability of insulin-sensitive tissues to respond appropriately to insulin. Because insulin release and activity are essential processes for glucose homeostasis **(**Galicia-Garcia et al., 2020**)**. T2-DM comprises 80% to 90% of all cases of diabetes mellitus which are responsible for significant mortality **(**Gupta et al., 1978**).** The chronic complications of diabetes are broadly divided into microvascular and macrovascular, with the former having much higher prevalence than the latter **(**Papatheodorou et al., 2018). The long–term effects of T2-DM include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation. There are several settings for DM treatment, however, these agents have some sort of side effects on different vital organs (Karalliedde et al., 2016; Pushpani et al., 2016). Metformin (Met) is considered one of the best choices of T2-DM treatments however, it may cause hyperthyroidism and metabolic acidosis (Quaile et al., 2010; Hu et al., 2017). Among these drugs Due to this regard, researchers are continuously trying to find anti-T2-DM agents with high efficacy and low side effects for the management and treatment of diabetes.

Over 2000 scorpion species have been described all over the world. Most of the scorpion species that are dangerous to humans belong to the family Buthidae including *Leiurus quinquestratus*, but some species in the families Scorpionidae and Hemiscorpiidae have also been classified as harmful **(**Laustsen et al., 2016; Lourenço, 2018**)**. The use of venom as therapeutic agents has been reported previously (Ghosh et al., 2019**)**. The scorpion venom is soluble in water, with a pH of neutral to alkaline. It is composed of oligopeptides, nucleotides, amino acids, and other organic components. It also contains enzymes such as phospholipases, hyaluronidase, and low-molecular weight compounds such as serotonin, histamine, protease inhibitors and histamine releasers. The potential therapeutic value of different scorpion venom compounds is being increasingly investigated, as these compounds may represent promising leads for the development of new pharmaceuticals for instance, anti-leishmanial, anti-malarial, anti-arthritic, anti-inflammation, anti-cancer, and antibacterial effects **(**Nipate et al., 2014; Uzair et al., 2018; Salama and El-Naggar, 2021; Mahmoud et al., 2021**)**. Previous study reported the effects of *Androctonus Crassicauda* scorpion venom in T1-DM in animal models **(**Roudbari and Imani, 2012**)**. Furthermore, the scorpion venom has active polypeptide that shortens wound healing with a stronger anti-inflammation and antibacterial effect and could be an effective topical drug for the treatment of T2-DM ulcers **(**Wan et al., 2017**)**. Interestingly, previous study reported that the whole-body extract of *Scorpio maurus palmatus* scorpion significantly increased the number of β-cells and the size of pancreatic islets in T2-DM mice, also it has antioxidant and regeneration capacity **(**Abdel-Rahman et al*.,* 2019**)**. *L. quinquestratus* is one of the dangerous scorpions around the world that represents a health hazard in Egypt. *L. quinquestratus'* venom (LQV) is composed of neurotoxic peptides, enzyme inhibitors, muco-polysaccharides, serotonin, hyaluronidase, phospholipase, and histamine **(**Ahmadi et al., 2020). The pharmacological activities of LQV have been evaluated for its antibacterial and anticancer effects (Nipate et al., 2014; Uzair et al., 2018**)**. Based upon our recent previous study which reported that the potential effect of LQV as anti T2-DM agent, this study aimed to optimize the therapeutic dose of LQV in T2-DM rats.

2. Materials and methods

Chemicals

Streptozotocin (STZ) was purchased from Sigma-Aldrich. Glucose, alpha amylase, aspartate amino transferase (AST), alanine amino transferase (ALT), and superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) kits were purchased from Bio diagnostic Company, Egypt. Phosphate buffer saline (PBS), ethanol and other chemicals were purchased from Al-Gomhoria Company, Tanta, Egypt. Metformin (Met) was purchased from local pharmacy in Tanta, Egypt, diluted by PBS and the concentration was adjusted to (150 mg/kg) b.

wt. in 300 µl for oral administration (Salemi et al., 2016).

Scorpion collection and venom preparation

One hundred *L. quinquestriatus* scorpions were collected from Aswan, Egypt by professional hunters. Scorpions were collected in plastic containers. Scorpions were transferred to Invertebrate Division, Zoology Department, Faculty of Science, Tanta University, Egypt. Then scorpion specimens were authenticated and identified by a specialist in animal taxonomy. According to the methodology of Abdel-Rahman et al. (2013), scorpions were milked using electrical stimulation (12-17V) of their telsons, and then venom (LQV) was lyophilized in corporate serum and vaccine; VACSERA Egypt. Different concentrations were prepared from the lyophilized venom. Sublethal doses were prepared according to Salama, (2014).

Experimental animals

Ninety-four adult male Sprague Dawley rats weighing between (120 ± 5) g and 8 weeks of age were purchased from animal husbandry, Alexandria University; rats were transported to Faculty of Science, Tanta University. The rats were given free access to normal diet and water and housed in cages at room temperature (25˚C) with a fixed 12 h light/dark cycle. All animal experiments were approved by guidelines of Institutional Animal Ethical Committee (IAEC) and conducted in accordance with ethical standards that approved at Zoology Department, Faculty of Science, Tanta University. The experimental animals used after acclimatization for two weeks before the beginning of the experiment.

Normal basal and high fat diets

Normal basal diet (NBD) is composed of protein 21%, fat 3.2% carbohydrate 68.2, and fibers 3.44%, according to the nutrient requirements of laboratory animals (NRC, 1995). HFD for induction of obesity: 10 % protein, 30% fat, 54.4 % carbohydrates, 3.5 % mineral mixture, 1 % vitamin mixture, 0.1% methionine and 1 % fiber (Altunkaynak and B.Z., 2005).

Experimental protocols

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

A total number of 36 male Sprague Dawley rats (120 ± 5) were divided into six groups (N = 6). These groups were injected with a single dose of (0.1-5 mg/kg) of LQV intraperitoneally (i.p) and were monitored for 24 hours to assess the $LD_{50.}$ This value was calculated using the probit analysis. Rats were monitored for 24 hrs to assess the $LD_{50.}$ This value was calculated using the probit analysis (Finney and Stevens, 1948).

Induction of T2-DM in rats

Rats were fasted overnight and then injected i.p with a single dose of STZ as 30 mg/kg (Guo et al., 2011). Because of the ability of STZ to induce fatal hypoglycemia because of massive pancreatic insulin release, the rats were provided with 10% glucose solution after 6 h of STZ administration for the next 48 hours to prevent hypoglycemia (Palsamy and Subramanian, 2008). Seven days following the STZ injection, blood was drawn from the tail vein and glucose concentration was measured using a portable glucometer (One Touch Select, Life scan, Inc., CA. USA). Animals with a FBS concentration >350 mg/dl were included in this study as T2-DM rats (T2-DM rats).

Experimental groups

Sixty-four rats were divided into 8 groups (N = 8 per group). Gp1 (Ctrl): Normal rats fed on chow diet. Gp2 (T2-DM): T2-DM rats with no treatments. Gp3 (D.+ Met): T2-DM rats had administered with Met Gp4 (D. + 1/10 LD_{50}): T-2DM rats had administered with 1/10 of LQV. Gp5 (D. + 1/40 LD_{50}): T-2DM rats had administered with 1/40 of LQV. Gp6 (D. + 1/80 LD_{50}): T-2DM rats had administered with 1/80 of LQV. Gp7 (D. + 1/160 LD_{50}): T-2DM rats had administered with 1/160 of LQV. Gp8 (D. + 1/320 LD_{50}): T-2DM rats had administered with 1/320 of LQV (Fig. 1).

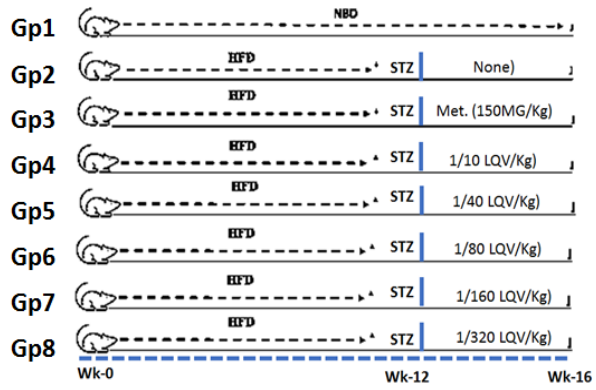

Fig. 1. The timeline and experimental design of the study

Determination of total body weight change, absolute and relative organs weights

All groups of rats were weighed at the beginning of treatment in week 12 (I. b. wt) and at the end of the experiment in week 16 (F. b. wt). The percentage of the change in the total body weight was calculated as follows: (I. b. wt – F. b. wt / I. b. wt) × 100. Also, percentages of absolute and relative organ weights (organ wt. /b.wt × 100) of all rats were taken after organs have been necropsied.

Determination of hematological and biochemical parameters

Blood samples were collected from arterial blood vessels and heart chambers then sera were separated by centrifugation for biochemical analysis. Serum glucose and alpha-amylase were determined according to Tietz, (1995) and Jones and Hattersley, (2013). Serum ALT and AST activities were determined as described by Thomas, (1998) and Rei, (1984). Serum total protein was determined as described by Tietz, (1994). Kidney functions (urea and creatinine) were determined as described by Thomas, (1998) and Newman and Price, (1999). SOD activity was determined as described by Nishikimi et al. (1972). CAT activities were measured following the methodology of Aebi, (1984). MDA was assessed based on the methods that have been previously described by Li and Chow, (1994).

Statistical analysis

All data are the means of three replicates. Normality of the data was tested with the Kolmogorov-Smirnov test. A two-way analysis of variance (ANOVA) was used to test the effect of pollution with heavy metals on hemocytes and hemolymph of the polluted and depurated crayfish sites (2 levels). If there is a significant difference between means, Tukey's post-hoc comparisons among different groups were performed. P-values.

3. Results

The acute LD_{50} value of LQV in rats

The phytochemical analysis showed that the TPC of GPE was 4.7 ± 0.13, while this value to determine the median lethal dose of the LQV that killed 50% of rats (LD_{50}), the probit analysis was used. Five groups (5 rats/each) were injected intraperitoneal (i.p) with different doses of the LQV for 24 hours. The LD_{50} values of LQV that killed 50% of rats was 0.29 mg/kg b.wt (Fig. 2).

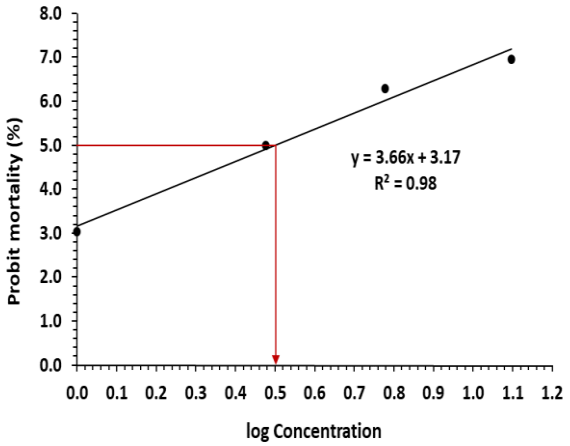

Fig. 2. The median lethal dose (LD_{50}) of LQV on rats after 24 hours using probit analysis.

Effect of the treatment with Met or with different doses of LQV on the percentages of body weight changes

The results showed that the percentages of body weight (% b.wt) changes in T2-DM rats was increased significantly when compared to the negative control group ($P < 0.05$). Treatment of T2-DM rats with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} the % b.wt changes when compared to T2-DM rats alone ($P < 0.05$). However, treatment with 1/160, or 1/320 did not show significant changes in the % b.wt change when compared to T2-DM rats alone (Table 1). The relative liver weight (RLW) of T2-DM rats were significantly increased when compared to the negative control group ($P < 0.05$). However, the RLW of T2-DM rats that were treated with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} were

significantly decreased when compared to their values of T2-DM rats alone ($P < 0.05$). Treatment of T2-DM rats with 1/160 or 1/320 of LQV LD_{50} did not show significant changes in RLW when compared to T2-DM rats alone (Table 1).

Kinetic measurement of glucose level in the different groups

The blood glucose levels were determined weekly in all groups under the study. The results showed that there was a significant increase in the level of glucose in the T2-DM group when compared to control group ($P < 0.05$). However, the treatment of T2-DM groups with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} led to a significant decrease in the blood glucose levels when compared to T2-DM rats alone. In addition, treatment of T2-DM rats with 1/160, or 1/320 of LQV LD_{50} did not show significant change in the blood glucose level when compared to T2-DM rats alone (Fig. 3)

Effects of the treatment with Met or with different doses of LQV on the serum α-amylase activity in the different groups

The results showed that α-amylase activity was significantly decreased in the T2-DM group when compared to their value in the control group ($P \leq 0.05$). The treatment of T2-DM groups with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} showed significant decrease in the α-amylase activity when compared to their values in the T2-DM group alone ($P \leq 0.05$). Treatment of T2-DM groups, however, with 1/160, or 1/320 of LQV LD_{50}, did not show significant changes in the α-amylase activity when compared to T2-DM rats alone ($P \leq 0.05$) (Fig. 4).

Effects of the treatment with Met or with different doses of LQV on the hematological parameters

The results showed that red blood cells (R.B.Cs) count, hemoglobin (Hb) concentration, and hematocrit (Hct) percentages were significantly decreased in the T2-DM group when compared to their control group ($P < 0.05$). As compared to the control groups, the platelets count was significantly decreased in the T2-DM group ($376.35 \pm 33.3 \times 10^3/\mu L$) ($P < 0.05$). The treatment of T2-DM groups with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} led to improvement in the alterations that were induced in the above-mentioned hematological parameters of T2-DM groups. The treatment of T2-DM groups with 1/160, or 1/320 of LQV LD_{50} did not show significant changes in the values of R.B.Cs count, hemoglobin (Hb) concentration, hematocrit (Hct) percentages, and platelets count when compared to T2-DM rats alone ($P \geq 0.05$) (Table 2a**)**. The results showed that white blood cells (W.B.Cs) count was significantly decreased in the T2-DM group when compared to their control values ($P \geq 0.05$). In the T2-DM group, monocytes and neutrophiles percentages were significantly increased; however, lymphocytes percentages were decreased when compared to negative control. The treatment of T2-DM groups with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} restored in the alterations that were induced in the leucocytes count of T2-DM groups. The treatment of T2-DM groups with 1/160, or 1/320 of LQV LD_{50} did not show significant changes in the values of W.B.Cs count when compared to T2-DM rats alone ($P \geq 0.05$) **(**Table 2b**)**.

Effects of the treatment with Met or with different doses of LQV on the liver function in the different groups

The results showed that the liver transaminase activities of ALT and AST were increased significantly in the T2-DM group when compared to their values in the control group ($P < 0.05$). Treatment of T2-DM groups with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} led to a significant decrease in the activities of ALT and AST when compared to the T2-DM group alone. The treatment of T2-DM groups, however, with 1/160, or 1/320 of LQV LD_{50} did not show significant changes in the activities of ALT and AST when compared to T2-DM rats alone (Table 3).

The results showed that activities of alkaline phosphatase (ALP) were increased significantly in the T2-DM group when compared to their values in the control group ($P < 0.05$). Treatment of T2-DM groups with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} led to a significant decrease in the activities of ALP

when compared to the T2-DM group alone. The treatment of T2-DM groups, however, with 1/160, or 1/320 of LQV LD_{50} did not show significant changes in the activities of ALP when compared to T2-DM rats alone ($P < 0.05$) (Table 3).

Effects of the treatment with Met or with different doses of LQV on the oxidative stress biomarkers

The results showed that malondialdehyde (MDA) level was increased significantly in the T2-DM group when compared to their values in the control group ($P \leq 0.05$). Treatment of T2-DM groups with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} led to a significant decrease in the MDA level when compared to the T2-DM group alone. The treatment of T2-DM groups, however, with 1/160, or 1/320 of LQV LD_{50} did not show significant changes in the MDA level when compared to T2-DM rats alone ($P \leq 0.05$) (Table 4). The results showed that activities of superoxide dismutase (SOD) and catalase (CAT) were decreased significantly in the T2-DM group when compared to their values in the control group ($P \leq 0.05$). Treatment of T2-DM groups with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} led to a significant increase in the SOD and CAT activities when compared to the T2-DM group alone. Treatment of T2-DM group, however, with 1/160, or 1/320 of LQV LD_{50} did not show significant changes in the activities of SOD and CAT when compared to T2-DM rats alone ($P \leq 0.05$) (Table 4).

Histopathological investigations

Examination of hematoxylin and eosin-stained hepatic sections from the control group showed intact hepatocytes distributed in an ordered fashion and displayed a typical hepatic architecture, including organized hepatocytic cords radiating from normal central vein, normal hepatocyte morphology, with normal blood sinusoid lined with Kufpper cells (Fig. 5A). Hepatic sections from the T2-DM rats stained with H&E exhibited distortion of the liver architecture with dilated irregular central vein and degenerated endothelium **(**Fig. 5B**)**. Mononuclear cellular leucocytic infiltration is present around the central vein. The blood sinusoids dilated in many areas with enlarged Kupffer cells. Some hepatocytes are vacuolated while others are pale hepatocytes. Presence of pyknotic cells.

In T2-DM groups treated with metformin, hepatic sections displayed a very little amelioration in their histological structure (Fig. 5C).

Liver sections showed disarrangement of the hepatic strands with vacuolated hepatocytes. The central vein appears dilated with disintegrated endothelium and mononuclear cellular infiltration around it. The presence of dilated blood sinusoids in many areas. In T2-DM rats treated with 1/10 of LQV LD_{50} of, the histological architecture of liver exhibited a weak amelioration (Fig. 5D). Furthermore, presence of distortion of the hepatic cords with dilated central vein and blood sinusoids. Abnormal hepatocytes with some hepatocytes are vacuolated cytoplasm and marginal nuclei. The pyknotic area is still present. Treatment of T2-DM rats treated with 1/40 LQV LD_{50} showed a considerable improvement in the histological structure of the liver sections. In further details, the hepatocytes appeared with normal structure arranged in normal stands radiating from normal-sized central vein. Whereas, presence of some degenerated endothelial cells, dilated blood sinusoids and leucocytic infiltration (Fig. 6A). The hepatic sections from T2-DM rats treated with 1/80 of LQV LD_{50} displayed obvious recovery in their histological structure including well-organization of the hepatic strands radiating from normal central vein with intact endothelium, intact hepatocytes with vesicular nuclei. However, dilated blood sinusoids and vacuolated hepatocytes are still present in some areas (Fig. 6B). Liver sections from T2-DM rats treated with 1/160 LD_{50} or with 1/320 LD_{50} of LQV showed various degrees of histopathological signs. Such signs were represented by dilated congested central vein with degenerated endothelium. The blood sinusoids appear dilated in many areas. Some hepatocytes are paled and vacuolated. Presence of pyknotic area and cellular leucocytic infiltration (Fig. 6C and D).

Table 1. The % of body weigh change and relative liver weigh change

Groups	% of b.wt change	RLW (%)
Ctrl	44.2%	3.38 ± 0.17 [a]
D. alone	29.4%	.03 ± 0.1 [b ع]
D + Met	22.8%	3.36 ± 0.15 [a]
D + 1/10 LD_{50}	19.4%	3.35 ± 0.21 [a]
D + 1/40 LD_{50}	20.1%	3.4 ± 0.36 [a]
D + 1/80 LD_{50}	18.6%	2.63 ± 0.12 [b]
D + 1/160 LD_{50}	25.4%	2.63 ± 0.12 [b]
D + 1/320 LD_{50}	27.4%	3.35 ± 0.21 [a]

The values represented mean ± SD. Metformin; **LD_{50}:** Median lethal dose (50%); *P*-value < 0.05 was statistically significant.

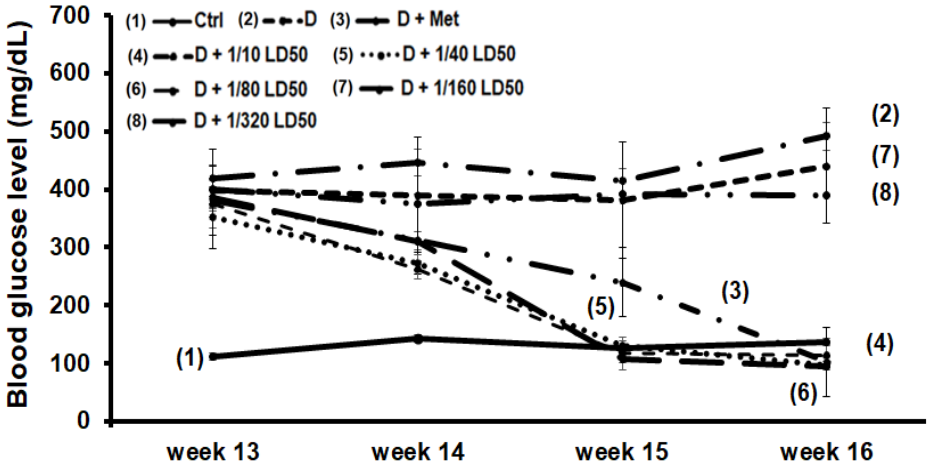

Fig. 3. Kinetic changes in the glucose levels among the different groups under the study under different treatment protocols. The values represented mean ± SD. Ctrl: Control group; D: Diabetic; Met: Metformin; LD_{50}: The medial lethal dose (50%). *P*-value < 0.05 was statistically significant.

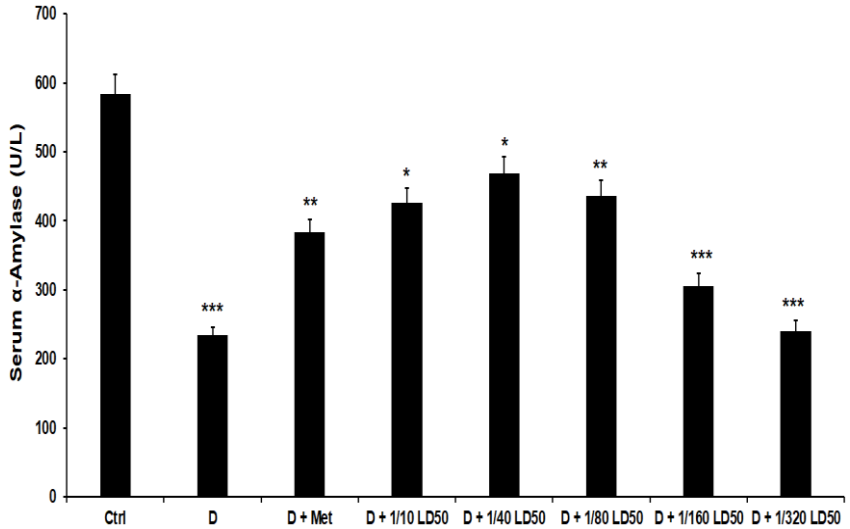

Fig. 4. α-Amylase level in the different groups under the study. The values represented mean ± SD. **Ctrl:** Control group; **D:** Diabetic; **Met:** Metformin; **LD_{50}:** The median lethal dose (50%). **P*-value < 0.05 was statistically significant

Table 2a. The total count of R.B.Cs, Hb concentration, Hct values, and the total platelets count of different

Groups	R.B.Cs ($\times10^6$/µL)	Hb (g/dL)	Platelets ($\times10^3$/µL)
Ctrl	6.19 ± 0.45	11.27 ± 0.83	654.75 ± 119.9
D. alone	3.25 ± 0.28*	7.05 ± 0.60*	376.35 ± 33.3*
D + Met	7.33 ± 0.48*	13.12 ± 0.63	754.25 ± 86.3
D + 1/10 LD_{50}	7.75 ± 0.28*	13.55 ± 0.3	812.25 ± 113.5
D + 1/40 LD_{50}	6.46 ± 1.35	11.87 ± 2.5	606.75 ± 121.3
D + 1/80 LD_{50}	8.16 ±0.52*	15.43 ± 1.06*	778.5 ± 82.6
D + 1/160 LD_{50}	4.62 ±0.27*	12.32 ± 0.83	464.72 ± 85.7
D + 1/320 LD_{50}	4.18 ± 0.03*	12.22 ± 0.36*	412.25 ± 42.8

The values represented mean ± SD. **R.B.Cs:** Red blood cells; **Hb: Hemoglobin; Ctrl:** Control group; **D:** Diabetic; **Met:** Metformin; **LD_{50}:** Lethal dose (50%). *P*-value < 0.05 was statistically significant.

Table 2b. The total count of white blood cells and their differential percentages

Groups	W.B.Cs ($\times10^3$/µL)	Monocytes (%)	Lymphocytes (%)	Neutrophils (%)
Ctrl	12.87 ± 1.04	4.27 ± 0.59	78.2 ± 2.26	17.25 ± 0.85
D. alone	6.80 ± 0.65*	9.13 ± 0.32*	59.92 ± 3.86*	30.80 ± 0.76*
D + Met	11.38 ± 2.1	6.71 ± 0.88	70.25 ± 2.57	23.05 ± 1.7*
D + 1/10 LD_{50}	10.51 ± 0.98	6.62 ± 1.49	71.70 ± 13.55	20.71 ± 0.34*
D + 1/40 LD_{50}	11.32 ± 3.7	7.32 ± 1.54	69.55 ± 4.2	21.25 ± 2.67*
D + 1/80 LD_{50}	11.74 ± 2.05	7.40 ± 1.06	68.75 ± 8.16	22.35 ± 6.13
D + 1/160 LD_{50}	7.82 ± 1.99	5.35 ± 1.08	51.12 ± 1.56	28.65 ± 1.2*
D + 1/320 LD_{50}	4.70 ± 0.84*	4.37 ± 2.56	55.82 ± 3.82	29.95 ± 1.34

The values represented mean ± SD. **R.B.Cs:** Red blood cells; **Hb: Hemoglobin; Ctrl:** Control group; **D:** Diabetic; **Met:** Metformin; **LD_{50}:** Lethal dose (50%). *P*-value **P* < 0.05 was statistically significant.

Table 3. Serum ALT, AST, and ALP activities in the different groups.

Groups	ALT(U/L)	AST (U/L)	ALP (U/L)
Ctrl	30.75 ± 1.31	80.39 ± 3.14	134.25 ± 4.97
D. alone	79.25 ± 3.76***	192.5 ± 4.88***	277.25 ± 8.53***
D + Met	49.32 ± 2.38**	99.18 ± 2.87	164.25 ± 3.98**
D + 1/10 LD_{50}	47.68 ± 2.65**	110.59 ± 3.44*	156.37 ± 5.16*
D + 1/40 LD_{50}	40.75 ± 2.15*	106.71 ± 3.27*	154.29 ± 6.02*
D + 1/80 LD_{50}	44.21 ± 2.37**	114.28 ± 4.41**	144.67 ± 5.90*
D + 1/160 LD_{50}	58.54 ± 2.95**	158.63 ± 4.90***	213.78 ± 6.43***
D + 1/320 LD_{50}	69.43 ± 3.07***	169.22 ± 5.07***	239.47 ± 5.19***

The values represented mean ± SD. **Ctrl:** Control group; **D:** Diabetic; **Met:** Metformin; **LD_{50}:** Lethal dose (50%); **ALT:** Alanine transaminase; **AST:** Aspartate transaminase; **ALP:** Alkaline phosphatase. *P*-value < 0.05 was statistically significant.

Table 4. Superoxide dismutase, catalase activities, and malondialdehyde levels

Groups	MDA (nmol/mg tissue)	SOD (U/mg tissue)	CAT (U/mg tissue)
Ctrl	33.25 ± 1.81	11.52 ± 0.87	85.89 ± 4.68
D. alone	80.87 ± 3.53***	5.56 ± 0.43***	52.34 ± 3.56***
D + Met	49.92 ± 2.15**	7.51 ± 0.47**	70.85 ± 41.56**
D + 1/10 LD_{50}	41.88 ± 2.48**	8.26 ± 0.57**	72.75 ± 4.58**
D + 1/40 LD_{50}	45.57 ± 1.92**	9.54 ± 0.09*	75.2 ± 3.89*
D + 1/80 LD_{50}	47.46 ± 3.68**	8.33 ± 0.44**	71.87 ± 4.07**
D + 1/160 LD_{50}	62.49 ± 3.07***	7.18 ± 0.76***	63.98 ± 4.91***
D + 1/320 LD_{50}	74.54 ± 3.45***	6.03 ± 0.32***	58.47 ± 3.71**

The values represented mean ± SD. **Ctrl:** Control group; **D:** Diabetic; **Met:** Metformin; **LD_{50}:** Lethal dose (50%); **MDA:** malondialdehyde; **SOD:** Superoxide dismutase; **CAT:** Catalase. *P*-value < 0.05 was considered to be statistically significant.

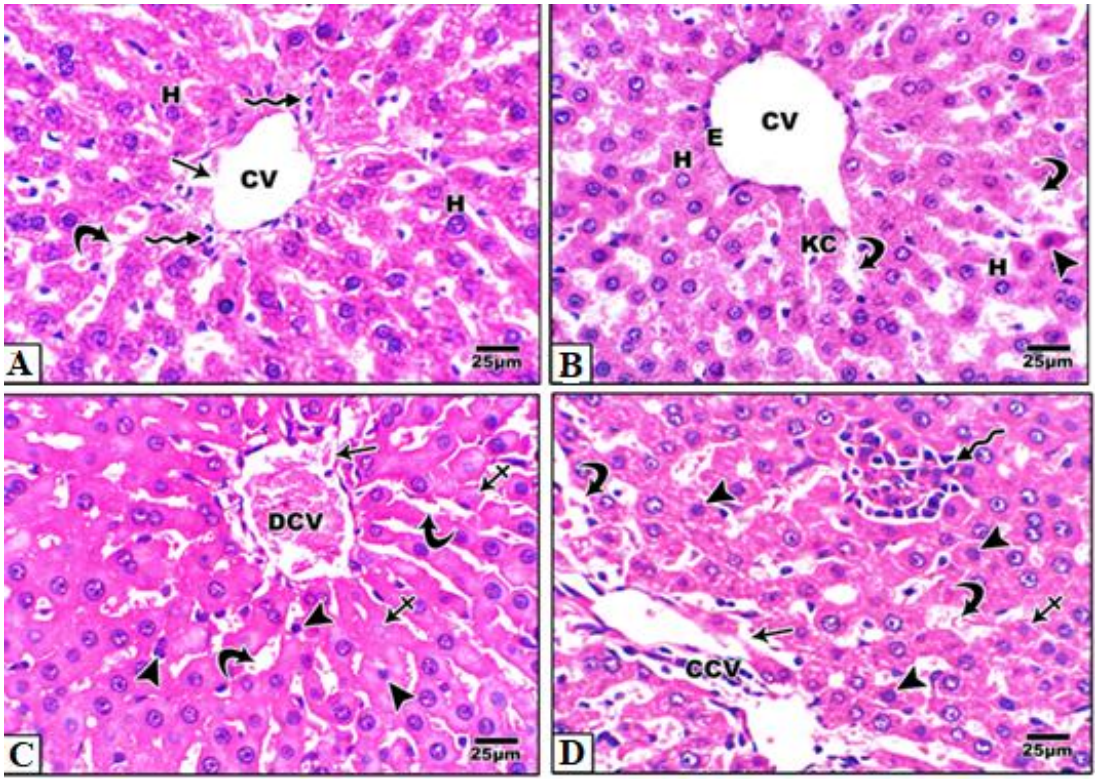

Fig. 5. Photomicrograph of liver sections from control, diabetic, diabetic/Met and diabetic+1/10 LQV LD_{50} groups stained with H&E. A) Liver section of control rats stained with H&E showing central vein (CV) lined with endothelial cells (E), polyhedral hepatocytes (H), and the blood sinusoids (BS) lined with Kupffer cells (KC) X=100. **B**) liver sections of diabetic rats showed dilated central vein (DCV) with degenerated endothelium (arrow), blood sinusoids (curved arrows) with enlarged Kupffer cells (thick arrow), vacuolated (tailed arrow) and pale hepatocytes (crossed arrow), pyknotic area (arrow head), and necrotic area (zigzag arrows) X=400. **C)** Liver sections of diabetic/Met showing dilated central vein (DCV) and blood sinusoids (curved arrows) with degenerated endothelium (arrow), vacuolated hepatocytes (tailed arrow), pyknotic area (arrow head), and necrotic area. (zigzag arrows) X= 400 **D)** Liver sections of diabetic +1/10 LD_{50} of *LQV* showed dilated central vein (DCV) and blood sinusoids (curved arrows), hepatocytes are vacuolated (tailed arrow), and pyknotic area (arrow head) X= 400.

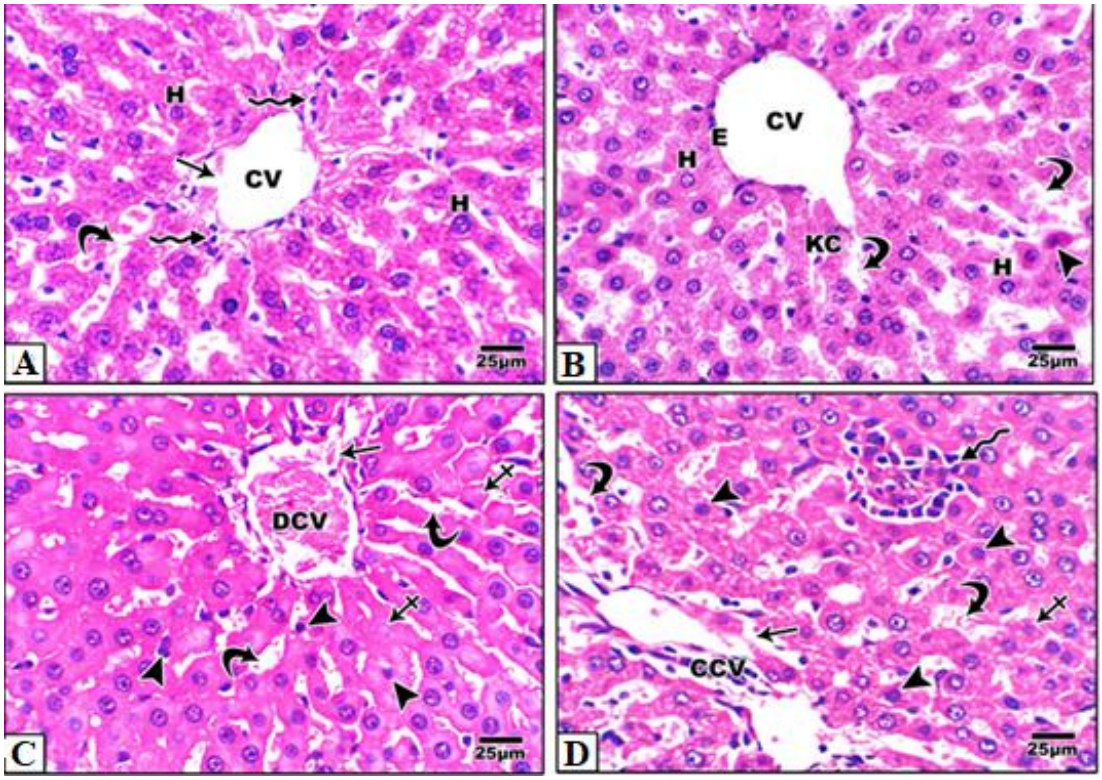

Fig. 6. Photomicrograph of liver sections from the diabetic rats treated with 1/40, 1/80. 1/160 and 1/320 LQV LD_{50}. **A**) Liver section of diabetic +1/40 LD_{50} LQV showing normal hepatocytes (H) central vein (CV) with degenerated endothelium (arrow), dilated blood sinusoids (curved arrows), necrotic area (zigzag arrows). **B**) Liver sections of diabetic +1/80 LD_{50} showing central vein (CV) lined with flat endothelial cells (E), normal hepatocytes (H), dilated blood sinusoids (curved arrows), and pyknotic area (arrow head). **C**) Liver sections of diabetic +1/160 LD_{50} showing dilated central vein (DCV) with degenerated endothelium (arrow), dilated blood sinusoids (curved arrows) with enlarged Kupffer cells (thick arrow), vacuolated hepatocytes (tailed arrow), pale hepatocytes (crossed arrow), pyknotic area (arrow head), necrotic area (zigzag arrows). D). Liver sections of diabetic + 1/320 LD_{50} showing congested central vein (CCV) with degenerated endothelium (arrow), dilated blood sinusoids (curved arrows) with enlarged Kupffer cells (thick arrow), vacuolated hepatocytes (tailed arrow), pale hepatocytes (crossed arrow), pyknotic area (arrow head), necrotic area (zigzag arrows) X=400

4. Discussion

Treatment of T2-DM with antidiabetic drugs led to several side effects such as hypoglycemia, headache, dizziness, nausea, and hypersensitivity reactions. Therefore, finding new strategies for diabetic management is necessary and new drugs in clinical trials and global sales (Dahlén et al., 2022). The therapeutic potential of venom-derived drugs is evident today. The success of venom-derived compounds is linked to their increased bioactivity, specificity, and stability (Coulter-Parkhill et al., 2021). *L. quinquestriatus* represents one of the most dangerous species of scorpions all over the world and it is encountered in Egypt. Scorpion venoms are rich bioactive peptide libraries that offer promising molecules that may lead to the development of new drugs, which can be used for the treatment of various diseases. The LQV and their peptides showed anticancer, antimicrobial, antiviral, anti-nociception, and anti-inflammation (Hmed et al., 2013; Akef, 2019; El Hidan et al., 2021). Abdel-Rahman et al. (2019) reported the antidiabetic effect of the scorpion Scorpio maurus palmatus body extract in alloxan-induced diabetic mice model. The present study was extended to optimize the appropriate dose that could treat T2-DM in rats by using different doses of LQV LD_{50} include 1/10, 1/40, 1/80, 1/160, or 1/320 of LQV LD_{50}. The LD_{50} values of LQV in the present work evaluated as 0.3 mg/kg b.wt. A previous study was used to determine the LD_{50} of the

Hottentotta saulcyi (Scorpiones, Buthidae) scorpion venom was 0.73 mg/kg in mice (Yağmur et al., 2015). Furthermore, a previous study of Salama, (2013) used different doses of LQV and investigated the LD_{50} of LQV in males and female and weanling albino mice and reported that adult female mice were around two times more sensitive to the venom (LD_{50} = 0.09 µg/g of body weight) than male (LD_{50} = 0.2 µg/g of body weight). A previous study reported the LD_{50} of *Mesobuthus eupeus* venom as 6.95 mg/kg (Khoobdel et al., 2013).

The group of T2-DM rat showed a significant decrease in the % b.wt change when compared to the negative control. Previous study reported that after STZ injection in rats and development of T2-DM there was significant increase in the body weight in diabetic group in comparison with control group, this could be due to increased food intake, increased activity of the leptin receptor, impaired glucose utilization and increase energy storage (Kotb et al., 2022). A previous study showed a significant increase in body weight of high fat diet fed animals in comparison with control group. This occurs because of increased satiety produced by high fat diet, the increased body weight is due to increased fat mass (Al-Qulaly et al., 2021). The % b.wt change of diabetic groups that were treated with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} was significantly improved when compared to the diabetic rats alone. Treatment of T2-DM group with different doses of LQV improved rat's body weight changes due to the improvement of carbohydrates and fats metabolic disorders that induced by diabetes. Met induces weight loss through activating lipolysis by inhibiting adipogenesis. It also inhibits carbohydrate absorption and bile salt uptake through stimulation of glucagon like peptide 1 (GLP-1) which inhibits energy production (Mobasher, 2021).

The current study showed that the relative liver weight (RLW) of T2-DM rats were significantly increased when compared to the negative control group. However, the RLW of T2-DM rats that were treated with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} were significantly decreased when compared to their values of T2-DM rats alone. Treatment of T2-DM rats with 1/160 or 1/320 of LQV LD_{50} did not show significant changes in RLW when compared to diabetic rats alone.

Diabetes-associated anemia has been reported due to the increased non-enzymatic glycosylation of R.B.Cs membrane proteins, which correlates with hyperglycemia (Mahmoud, 2013). Therefore, hematological parameters could be an important tool in the assessment of deleterious effect of antidiabetic drugs (Mansi and Lahham, 2008). The present study showed that the total R.B.Cs count, Hb concentration, Hct percentages, platelets count, and the total counts of W.B.Cs were significantly decreased in the T2-DM group. Previous study reported that there were decreases in the above-mentioned hematological parameters in the prediabetic and diabetic rats versus the control (Krisnamurti et al., 2022).

The treatment of T2-DM groups with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} led to improvement in these hematological parameters. Previous study evaluated the hematological parameters in rats injected with Tityus serrulatus scorpion venom, their results showed an increase on R.B.Cs count, Hb concentration, and Hct value (Cusinato et al., 2010). Following the treatment of T2-DM rats with Met, 1/10, 1/40, or 1/80 of LQV LD_{50}, the level of hematological parameters and their related indices were improved. This gives an indication that LQV can stimulate erythropoietin formation, which stimulates stem cells in the bone marrow to produce blood cells (Ohlsson and Aher, 2012).

The damaging effect of STZ on the pancreatic beta cells resulted in T2-DM rats that were evaluated by assessment of glucose levels. The results obtained from the current study showed that there was a significant increase in the level of glucose of the T2-DM group when compared to control group. However, the treatment of T2-DM groups with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} led to a significant decrease in the blood glucose levels when compared to T2-DM rats alone. Treatment of T2-DM rats with 1/160, or 1/320 of LQV LD_{50} did not show significant change in the blood glucose level when compared to T2-DM rats alone. These findings agreed with previous studies reported that Met decreases blood glucose level via

inhibition of intestinal glucose absorption, suppression hepatic glucose production, reduction in hepatic glucose output, and facilitation of glucose uptake by tissues and improving insulin sensitivity (Mahmood, 2021).

Met increases glucose uptake in skeletal muscle via increased translocation of GLUT-4 transporters to the plasma membrane and stimulating GLP-1. Furthermore, Met decreases blood glucose through increasing the activity of the insulin receptor and its substrate which increases the uptake of glucose in the liver cell (Gedawy et al., 2020; Ibrahim et al., 2023). Previous studies reported the therapeutic potential of scorpion venom-derived peptides in the improvement of diabetic status (Baile et al., 2019; Coulter-Parkhill et al., 2021).

The T2-DM has adverse effects on multiple organs, the physiological function of liver and kidneys was impaired in the persistent environment of hyperglycemia (Wu et al., 2023). In the current study, the T2-DM rats showed significant increase in the activities of serum ALT, AST, ALP, urea, and creatinine levels. This could be due to hepato-renal dysfunctions that were induced by diabetic complications. The treatment of T2-DM rats with 1/10, 1/40, or 1/80 of LQV LD_{50} led to improvement in the hepato-renal functions that is evidenced by significant decrease in serum ALT, AST, ALP activities, urea, and creatinine levels. This could be due to the ameliorative effect of LQV on the hepato-renal dysfunctions. These findings were in accordance with previous studies that reported that Met treatment improves the hepato-renal dysfunctions induced in T2-DM rats (Elwan et al., 2022). The treatment with 1/160, or 1/320 of LQV LD_{50} did not show significant changes in the activities of ALT and AST and kidneys functions parameters when compared to diabetic rats alone, this could be due to the very low concentrations.

The ameliorative effect of LQV on acetic acid-induced colitis in mice has been reported (Mahmoud et al., 2021). Furthermore, previous study reported the hepato- and nephroprotective effects of bradykinin potentiating factor from scorpion (Buthus occitanus) venom (Salman et al., 2016). Reactive oxygen species (ROS) and oxidative stress has been considered as a major hallmark for the pathogenesis and development of T2-DM. ROS are highly reactive species and almost all cellular components are chemically changed due to the influence of ROS that ultimately results in the production of lipid peroxidation. Lipid peroxidation is a major causative factor for the development of oxidative stress that leads to overt T2-DM and its associated micro- and macro-vascular complications. Diabetes mellitus, mechanisms involved in hyperglycemia-induced oxidative stress with particular focus on T2-DM (Oguntibeju, 2019). The results of this study reported that post STZ injection in T2-DM group a significant decreased the activities of SOD and CAT. In contrast, the level of MDA was significantly increased due to lipids peroxidation that was induced by oxidative stress in the T2-DM rats. Oxidative stress has long been considered as one of the main damaging factors responsible to induce various causative factors that are responsible for the development of insulin resistance, impaired insulin secretion from the β-cells of pancreatic islets and pathogenesis of T2-DM (Rehman and Akash, 2017). It has been evidenced from various experimental studies that total antioxidant status significantly decreases in T2-DM which indicated the decreased levels of antioxidant enzymatic such as SOD, GPX, and CAT. Previous study evaluated the oxidative stress in STZ-induced rodents and reported that in T2-DM rats there was significant reduction in SOD and CAT accompanied with increase in MDA level (Jamal Gilani et al., 2021).

Met can act in muscle, inhibiting the complex I of the electron transport chain and decreasing mitochondrial ROS. Oxidative stress and some biochemical alterations due to LQV in rats have been reported (Salman and Hammad, 2017). Treatment of T2-DM rats with Met or different doses of LQV led to improvement the antioxidant/oxidant hemostasis as the activities of SOD and CAT were significantly increased and MDA levels were significantly decreased when compared to the T2-DM rats only. These findings were in line with previous studies that reported the ameliorative effects of natural constitutes on oxidative stress induced by

diabetes mellitus in rats (Mobasher, 2021; Bayrak et al., 2022).

The correlation is well established between T2-DM and nonalcoholic fatty liver disease including a large continuum of liver disorders from steatosis to cirrhosis. Histological changes in the liver and kidneys of T2-DM rats have been reported (Bilal et al., 2016). In this study, histopathological investigations showed that the liver sections of the STZ-induced T2-DM rats showed necrotic areas. Interestingly, the treatment of T2-DM rats with Met, 1/10, 1/40, or 1/80 of LQV LD_{50} led to improvement in the hepato-renal histopathological alterations. The liver and kidneys sections showed partial improvements in their architectures, a recovery of few cells to normal form, and reduction of the accumulated fibers. These findings were in agreement with previous studies that reported the impacts of natural constitutes on the liver and kidneys histopathology of T2-DM group) Elshater et al., 2011). A very recent study reported that the treatment with LQV ameliorates the histopathological changes of T2-DM rats' splenic tissues (Salama et al., 2024).

Conclusion

Collectively, treatment with different doses of LQV between 1/10, and 1/80 of LQV LD_{50} significantly improved the diabetic status in rats. These doses ameliorated the alterations that were induced in the hematological, biochemical, and histopathological investigations of diabetic rats.

Conflict of Interest

The authors declared that there is no conflict of interest.

References

Abdel-Rahman M.A., Mohammed A.K., Ahmed S.H., Binnaser Y.S., Ismail M., and Abdel-Nabi I.M. (2019). Antidiabetic effect of the scorpion Scorpio maurus palmatus body extract using alloxan-induced diabetic mice model. Journal Of Taibah University Science. 13: 504–513.

Aebi H. (1984). Catalase in vitro. Methods Enzymol. 105: 121–126.

Ahmadi S., Knerr J.M., Argemi L., Bordon K.C.F., Pucca M.B., Cerni F.A., Arantes E.C., Çalışkan F., and Laustsen A.H. (2020). Scorpion venom: detriments and benefits. Biomedicines. 8(5):118.

Akef H.M. (2019). Anticancer and antimicrobial activities of scorpion venoms and their peptides. Toxin Rev. 38: 41–53.

Al-Qulaly M., Okasha M.A., and Hassan M.G. (2021). Effect of ginger and cinnamon on induced diabetes mellitus in adult male albino rats. Bulletin of Egyptian Society for Physiological Sciences. 41: 373–388.

Bailey C. (2019). Glucose-lowering therapies in type 2 diabetes: opportunities and challenges for peptides. Peptides. 100: 9–17.

Bayrak B., Koroglu P., Karabulut Bulan O., and Yanardag R. (2022). Metformin protects against diabetes-induced heart injury and dunning prostate cancer model. Human and Experimental Toxicology. 40: 297–309.

Bilal H.M., Riaz F., Munir K., Saqib A., and Sarwar M.R., (2016). Histological changes in the liver of diabetic rats: A review of pathogenesis of nonalcoholic fatty liver disease in type 1 diabetes mellitus. Cogent Medicine. 3: 1.

Coulter-Parkhill A., McClean S., Gault V.A., and Irwin N. (2021). Therapeutic Potential of Peptides Derived from Animal Venoms: Current Views and Emerging Drugs for Diabetes. Clin Med Insights Endocrinol Diabetes. 14: 11795514211006071.

Cusinato D., Souza A.M. Vasconcelos F., Guimarães L.F.L., Leite Flávia., Gregório Z.M.O., Giglio J.R., and Arantes E. (2010). Assessment of biochemical and hematological parameters in rats injected with *Tityus serrulatus* scorpion venom. Toxicon. 56: 1477–1486.

Dahlén A.D., Dashi G., Maslov I., Attwood M.M., Jonsson J., Trukhan V., and Schiöth H.B. (2022). Trends in Antidiabetic drug discovery: FDA approved drugs, new drugs in clinical trials and global sales. Front. Pharmacol. 12: 807548.

El Hidan M.A., Laaradia M.A., Hiba O.E, Draoui A., Aimrane A., and Kahime K. (2021): Scorpion-derived antiviral peptides with a special focus on medically important viruses: An update. BioMed Research International. 2021: 1-9. Article ID 9998420.

Elshater A.E., Salman, M., and Abd-Elhady, A. (2011). Physiological studies on the effect of a bradykinin potentiating factor (BPF) isolated from scorpion venom on the burnt skin of alloxan-induced diabetic Guinea pigs. Egypt. Acad. J. Biol. Sci. C Physiol. Mol. Biol. 3: 5–15.

Elwan M.M., El-Nahass E.E., Basyony M.A., Elshennawy E.O., and El-Naggar S.A. (2022). Metformin treatment improves the hepato-renal dysfunctions induced in Type-II diabetes in male rats. International Journal of Cancer and Biomedical Research. 6: 77–87.

Finney D.J., and Stevens W. L. (1948). A table for the calculation of working probits and weight in propit analysis. Biometrika 35(1-2):191-201.

Galicia-Garcia U., Benito-Vicente A., Jebari S, Larrea-Sebal A., Siddiqi H., Uribe K.B., Ostolaza H., and Martín C. (2020). Pathophysiology of type 2 diabetes mellitus. Int J Mol Sci. 21(17):6275.

Gedawy A., Al-Salami H., and Dass C.R. (2020). Role of metformin in various pathologies: State-of-the-art microcapsules for improving its pharmacokinetics. Therapeutic Delivery. 11: 733–753.

Ghosh A., Roy R., Nandi M., and Mukhopadhyay A. (2019). Scorpion venom–toxins that aid in drug development: A Review International Journal of Peptide Research and Therapeutics. 25: 27–37.

Guo H., Chi J., and Xing Y. (2011). Influence of folic acid on plasma homocysteine levels & arterial endothelial function in patients with unstable angina. Indian J. Med. Res. 129: 279–284.

Hmed B.N., Serria H.T., and Mounir Z.K. (2013). Scorpion peptides: potential use for new drug development. J Toxicol 2013: 958797.

Hu X, Liu Y, Wang C, Hou L, Zheng X, Xu Y, Ding L, Pang S. Metformin affects thyroid function in male rats. Oncotarget. 2017 Nov 20;8(64):107589-107595. doi: 10.18632/oncotarget.22536. PMID: 29296189; PMCID: PMC5746091.

Hu X, Liu Y, Wang C, Hou L, Zheng X, Xu Y, Ding L, Pang S. Metformin affects thyroid function in male rats. Oncotarget. 2017 Nov 20;8(64):107589-107595. doi: 10.18632/oncotarget.22536. PMID: 29296189; PMCID: PMC5746091.

Ibrahim Y., Fatma F., Elshymaa A., and Zahra S. (2023). Pathophysiological mechanisms of type 2 diabetes mellitus and the effects of metformin treatment in adult male albino rats. MJMR. 34: 209–214.

Jamal Gilani S., Nasser Bin-Jumah M., Al-Abbasi F.A., Shahid Nadeem M., Afzal M., Sayyed N., and Kazmi I. (2021). Fustin ameliorates hyperglycemia in streptozotocin induced type-2 diabetes via modulating glutathione/Superoxide dismutase/Catalase expressions, suppress lipid peroxidation and regulates histopathological changes. Saudi J Biol Sci. 28: 6963–6971.

Jones A.G., and Hattersley A.T. (2013). The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med. 30: 803–817.

Khoobdel M., Zahraei-Salehi T., Nayeri-Fasaei B., Khosravi M., Omidian Z., Motedayen M., and Akbari A. (2013): Purification of the Immunogenic Fractions and Determination of Toxicity in Mesobuthus eupeus (Scorpionida: Buthidae) Venom. Journal of arthropod-borne diseases. 7: 139–146.

Kotb A.M., Abdel-Hakim S.M., Ragy M.M., Elbassuoni E.A., and Abdel-Hakeem E.A. (2022). Metformin ameliorates diabetic cardiomyopathy in adult male albino rats in type 2 diabetes. Minia Journal of Medical Research. 33: 128–138.

Krisnamurti D.G.B., Purwaningsih E.H., Tarigan T.J.E., Soetikno V., and Louisa M. (2022). Hematological indices and their correlation with glucose control parameters in a prediabetic rat model. Vet World. 15: 672–678.

Kumar R.V. and Sinha V.R. (2012). Newer insights into the drug delivery approaches of alpha-glucosidase inhibitors. Expert Opin Drug Deliv. 9(4): 403–416.

Li X.Y., and Chow C.K. (1994). An improved method for the measurement of malondialdehyde in biological samples. Lipids. 29: 73–75.

Lourenço W.R. (2018). The evolution and distribution of noxious species of scorpions (Arachnida: Scorpiones) J. Venom. Anim. Toxins Incl Trop Dis. 2018: 24.

Mahmoud H.A., Salama W.M, Mariah R.A., and Eid A.M. (2021). Ameliorative effect of *Leiurus quinquestriatus* venom on acetic acid-induced colitis in mice. Scientific African. 14: e01009.

Mansi K., and Lahham J. (2008). Effects of *Artemisia sieberi* Besser (a. herba-alba) on heart rate and some hematological values in

normal and alloxan-induced diabetic rats. J Basic Appl Sci. 4: 57–62.

Marín-Peñalver JJ, Martín-Timón I, Sevillano-Collantes C, Del Cañizo-Gómez FJ. Update on the treatment of type 2 diabetes mellitus. World J Diabetes. 2016 Sep 15;7(17):354-95.

Mobasher M.A. (2021). Metformin: An AMPK-dependent antidiabetic drug with novel medical applications. International Journal of Cancer and Biomedical Research. 5: 1–12.

Newman D.J., and Price C.P. (1999). Renal function and nitrogen metabolites. In: Burtis CA, Ashwood ER (eds) Tietz textbook of clinical chemistry, 3rd edn. WB Saunders, Philadelphia. 1204–1270.

Nishikimi M., Rao N. A., and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun. 46: 849–853.

Oguntibeju O.O. (2019). Type 2 diabetes mellitus, oxidative stress, and inflammation: examining the links. Int. J. Physiol. Pathophysiol. Pharmacol. 11(3): 45–63.

Ohlsson A., and Aher S.M. (2012). Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Database Syst Rev. 9: CD004863.

Palsamy P., and Subramanian S. (2008). Resveratrol, a natural phytoalexin, normalizes hyperglycemia in streptozotocinnicotinamide induced experimental diabetic rats. Biomed Pharmacother. 47: 1–8.

Papatheodorou K., Banach M., Bekiari E., Rizzo M., and Edmond M. (2018). Complications of Diabetes 2017. Journal of Diabetes Research 2018: Article ID 3086167.

Pushpani M.C., Ranmalee E., and Kaarin J.A. (2016). The effect of diabetes medication on cognitive function: evidence from the PATH through life Study. BioMed Research International, Article ID 7208429.

Quaile MP, Melich DH, Jordan HL, Nold JB, Chism JP, Polli JW, Smith GA, Rhodes MC. Toxicity and toxicokinetics of metformin in rats. Toxicol Appl Pharmacol. 2010 Mar 15;243(3):340-7. doi: 10.1016/j.taap.2009.11.026. Epub 2010 Jan 13. PMID: 20004680

Quaile MP, Melich DH, Jordan HL, Nold JB, Chism JP, Polli JW, Smith GA, Rhodes MC. Toxicity and toxicokinetics of metformin in rats. Toxicol Appl Pharmacol. 2010 Mar 15;243(3):340-7. doi: 10.1016/j.taap.2009.11.026. Epub 2010 Jan 13. PMID: 20004680.

Rehman K., and Akash M.S. (2017). Mechanisms of inflammatory responses and development of insulin resistance: How are they interlinked? J Biomed Sci. 23 :87.

Roudbari L., and Imani S. (2012). The effects of Anderoctonus Crassicauda scorpion venom in the treatment of Diabetes Mellitus type 1 in Animal models Leila. Annals of Biological Research, 2012, 3 (12):5782–5785

Salama W. (2014). The Lethal Effect of *Leiurus Quinquestriatus* venom on adult and weanling albino mice with evaluating its effects on some biochemical parameters. Egyptian Journal of Zoology. 60: 429–441.

Salemi Z., Rafie E., Goodarzi M.T., and Ghaffari M.A. (2016). Effect of Metformin, acarbose and their combination on the serum level in nicotinamide/streptozocin-induced type 2 diabetic rats. Iran Red Crescent Med. J. 18(3): 23814.

Salman M.M., Kotb A.M., Haridy M.A., and Hammad S. (2016). Hepato- and nephroprotective effects of bradykinin potentiating factor from scorpion (*Buthus occitanus*) venom on mercuric chloride-treated rats. Excli J. 15: 807–816.

Sarkar B.K., Akter R., Das J., Das A., Modak P., Halder S., Sarkar A.P., and Kundu S.K. (2019). Diabetes mellitus: A comprehensive review. Journal of Pharmacognosy and Phytochemistry. 8(6): 2362–2371.

Singh N., Kesherwani R., Tiwari A.K., and Patel D.K. (2016). A review on diabetes mellitus. The Pharma Innovation. 5(7): 36–40

Thomas L. (1998). Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft. 27: 644–647.

Tietz N.W. (1995). Clinical guide to laboratory tests. 3rd Ed. Philadelphia: WB Saunders: 268–273.

Uzair B., Irshad S., Khan B., AzadB., Mahmood T., Rehman M., and Braga V. (2018). Scorpion venom peptides as a potential source for human drug candidates. Protein Peptide Letters. 25.

Wan T., Li L., Zhu Z., Liu S., Zhao Y., and Yu M. (2017). Scorpion Venom Active Polypeptide May Be a New External Drug of Diabetic Ulcer", Evidence-Based Complementary and

Alternative Medicine. 2017: Article ID 5161565. https://doi.org/10.1155/2017/5161565.

Wu T., Ding L., Andoh V., Zhang J., and Chen L. (2023). The mechanism of hyperglycemia-induced renal cell injury in diabetic nephropathy disease: An update. Life (Basel). 13(2): 539.

Yağmur E.A., Özkan Ö., and Karaer K.Z. (2015). Determination of the Median Lethal Dose and Electrophoretic Pattern of *Hottentotta saulcyi* (Scorpiones, Buthidae) Scorpion Venom. J Arthropod Borne Dis. 9: 238–245.