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The Anti-Diabetic Effect of *Rhopilema nomadica* Jellyfish Natural Extracts in Streptozoticin-Induced Type-2 Diabetes Mellitus in Rats.

Running title: The Hypoglycemic Effect of Jellyfish`s Natural Products in STZ-induced T2-DM Rat Model

Sabry A. El-Naggar [1]*, Wesam M. Salama[1]*, Nabila I. El-Desouki[1], Ghada A. Tabl[1], Rasha A. Abo Jobier[1], and Amal M. Abdelsattar[2]

[1]Zoology Department, Faculty of Science, Tanta University, Egypt

[2]Anatomy &Embryology Department, Faculty of Medicine, Tanta University

***Corresponding authors:**
Wesam M. Salama, Ph.D
Zoology Department, Faculty of Science, Tanta University, Egypt
E-mail: wesam.hassan@science.tanta.edu.eg
Mobile: (+2) 01200355329

Sabry Ali El-Naggar, Ph.D
Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt
E-mail: sabry.elnaggar@science.tanta.edu.eg
Mobile: (+2) 01068382357

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ABSTRACT

Globally, diabetes mellitus (DM) disease is one of the main causes of death. Type-2 diabetes mellitus (T2-DM) cannot be cured by first-line medications, and long-term use carries a significant risk of serious side effects. Novel antidiabetic agents with high efficacy are required. In the T2-DM rats' model, the anti-diabetic effect of *Rhopilema nomadica* venom (RNV) and *R. nomadica* umbrellar extracts (RNUE) was assessed. Biometric and biochemical measurements were determined. The median lethal doses (LD_{50}) of RNV and RNUE were estimated, and then Sprague Dawley male rats were split into five groups (n=10) as follows; group 1 (Gp1) served as a negative control. Gp2 to Gp5 were given a high-fat diet (HFD) and then, injected with STZ (30 mg/kg) interperitoneal (i.p.). Gp2 was kept as diabetic rats (T2-DM rats). Metformin (Met) (150 mg/kg), RNV (7.5 mg/kg), and RNUE (14.4 mg/kg) were given to Gp3, Gp4, and Gp5, respectively. All treatments were taken i.p., once every day for eight weeks body weight (b.wt) changes, the levels of glucose, C-peptide, biochemical parameters, and the histopathological alterations were assessed. *R. nomadica* diameter was 35- 40 cm, and its weight was 4- 4.5 kg. RNV and RNUE total antioxidant capacities were 2.39 ± 0.18 and 2.92 ± 0.26 mg/g. RNV and RNUE protein profiles showed eight and seven bands. Treatment of T2-DM rats with Met or RNUE led to a hypoglycemic effect as indicated by improvement of b.wt changes, glucose, C-peptide levels, hepato-renal function, antioxidant/oxidant biomarkers status, and insulin-secreting β-cells in the pancreatic islets.

Keywords: Anti-diabetic, *Rhopilema nomadica*, Type-2 diabetes mellitus, Umbrella extracts, Venom.

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INTRODUCTION

Diabetes mellitus (DM) is ranked among the top ten global causes of death (1). The current global DM population is over 462 million and this number could be increased to approximately 700 million by 2045. Middle-income countries are predicted to experience the largest relative increase in the prevalence of diabetes (21.1%), as opposed to high (12.2%) and low-income (11.9%) countries (2). Obesity is one of the most common diabetes risk factors, which contributes to the increase in its incidence (3). Generally, DM is linked to several serious diseases such as; peripheral neuropathy, nephropathy, cancer, micro-vascular problems, and cardiovascular disease (1,4). Type 1 diabetes (T1-DM), type 2 diabetes (T2-DM), and gestational diabetes (G-DM) are the three main types of the disease. Pancreatic islet beta cells (β-cells) are being destroyed, and insulin treatment is necessary for people suffering from T1-DM. While impaired insulin action or insufficient insulin secretion occurs in T2-DM. The variations in insulin levels that occur during pregnancy cause G-DM (5). T2-DM is characterized by insulin resistance (IR) and persistent hyperglycemia, therefore, insulin sensitizers, insulin secretagogues, alpha-glucosidase inhibitors, incretin-based therapies, and sodium-glucose cotransporter 2 inhibitors could be used for medications (6). Nowadays, first-line medications cannot treat T2-DM, and long-term use carries a significant risk of serious side effects, including fatal hypoglycemia and gastrointestinal reactions (1). For managing and treating diabetes, anti-diabetic medications with high efficacy and minimal side effects are needed (7).

Natural products are in use for developing therapeutics to treat DM (8). Marine organisms in particular have different potential bioactive ingredients that could be used as therapeutic agents for the treatment of several diseases (9,10). Different biological and pharmacological applications have been demonstrated for the bioactive compounds that are isolated from Cnidaria (11). For instance, components of *Pelagia noctiluca* venom were reported as anti-inflammatory and anticancer agents (12). Due to their nutritional value, jellyfish are eaten in China and Japan, where their consumption has been linked to a decline in the prevalence of coronary heart disease (13).

Rhopilema nomadica is a scyphozoan jellyfish belonging to the Rhizostomeae order and class Scyphozoa. It is the largest scyphomedusa recorded in Egypt's Mediterranean Sea (14). Previous research assessed the biological significance of several scyphomedusan jellyfish as an antioxidant, insecticidal, hypocholesterolemic, antifatigue, anticoagulant, anti-analgesic, antihemolytic, immune-simulative, antimicrobial, anti-cardiovascular disorder, and antihypertensive agents (10,15-17). To our knowledge, there are no reports available on the bioactivity of scyphomedusa to treat diabetes. Therefore, this study aimed to assess the anti-diabetic effect of natural products extracted from *R. nomadica* (venom and umbrella) that are widely distributed on the Egyptian coasts.

MATERIAL AND METHODS

Chemicals

Chemicals and reagents were bought from commercial chemical companies. Streptozotocin (STZ) was purchased from Sigma Company (USA). Metformin hydrochloride was purchased as tablets of 500 mg from MINAPHARM Pharmaceuticals Company (10th of Ramadan City, Egypt). Then, Met was dissolved in phosphate buffer saline (PBS) and adjusted to 150 mg/kg in 300 µl (18).

Glucose, C-peptide, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) kits were acquired from the Egyptian Bio-diagnostic Company (Dokki, Egypt).

Scyphomedusa collection

From July to August 2021, fifteen Scyphomedusa (Cnidaria, Scyphozoa, Rhizostomeae) specimens have been collected from the Mediterranean Sea at Alexandria's Sidi-Bishr station (31°15'54"N latitude

and 29°59'10"E longitude). Specimens were transported in tanks of cold seawater (5±2°C) to the Invertebrates lab, Zoology Department, Faculty of Science, Tanta University. The specimens were identified by a taxonomist.

Morphological measurements of *R. nomadica*

R. nomadica morphological structure was investigated, then the umbrella diameter and the body weight were determined. Immediately, the umbrella and the oral arms were quickly separated for further use.

Preparation of *R. nomadica* venom and extract

Rhopliema nomadica venom (RNV) was prepared by immersing the oral arms of R. nomadica in 1 L of 100% ethanol for 30 seconds at room temperature to allow the nematocysts to chemically discharge. After allowing the discharge to precipitate for 24 hours at 4°C, it was centrifuged for 30 minutes at 14,000 rpm. The precipitate was lyophilized and was kept as RNV at -80°C until needed (19). For the preparation of the *R. nomadica* umbrella extract (RNUE), the separated umbrella was cut into tiny (1 cm) pieces, soaked in 100% ethanol, and stored at room temperature. The dissolved materials in ethanol were centrifuged at 5000 rpm for 15 min and lyophilized to prepare RNUE. The powder was collected in clean tubes and stored at - 80ºC until further use (20).

Biochemical analysis and characterization of RNV and RNUE

The total protein of RNV and RNUE was estimated according to (21), lipids contents according to (22), and carbohydrates contents were calculated according to (23). Additionally, a protein profile using SDS–PAGE analysis was done (24). The DPPH scavenging activity was evaluated according to (25). The DPPH scavenging percentage has been calculated as follows: DPPH scavenging% = (Ao - As/Ao) X 100, where Ao is the absorbance of the blank and as is the absorbance of the sample at 515 nm. The median inhibitory concentration (IC50) necessary to lower the initial DPPH radical concentration by 50% was determined. According to (26), the total antioxidant capacity (TAC) was calculated.

Experimental animals

Rats (Sprague Dawley) with an average weight of 120 ± 4 g were obtained from animal husbandry, at Alexandria University. Animals were taken to Tanta University's Faculty of Science and held for a week for acclimation. The temperature and relative humidity were around 25 ± 1ºC and 55 ± 5%, respectively. The animals were treated following the animal care and use committee's ethical guidelines (protocol number IACUC-SCI-TU-0227).

The median lethal dose of RNV and RNUE determination

A number of seventy-two Sprague Dawley male rats were divided into twelve groups (n = 6). From group 1 (Gp1) to Gp6, rats were injected with different doses of RNV (125 to 1000 mg/kg b.wt). From Gp7 to Gp12, rats were injected with different doses of RNUE (200 to 2500 mg/kg b.wt). All doses were injected as a single dose via intraperitoneal (i.p) technique. Rats were monitored for 24 hours to assess the median lethal dose **(**LD_{50}). This value was calculated using the probit analysis (27).

Normal balanced and high-fat diet food components

The normal balanced diet (NBD) was used to feed healthy control rats. NBD contains 3% fat 21% protein, and 48.8% carbohydrates. The high-fat diet (HFD) contains 40 % fat 17% protein, and 43% carbohydrates (28).

Induction of T2-DM in rats

After 12 weeks of HFD feeding, Gp2 to Gp5 were given a single injection of STZ (30 mg/kg i.p.) (29). Blood samples were collected from the tail vein three days post-STZ injection. The glucose levels were determined using a portable glucometer (One Touch Select, Life Scan, Inc., California, USA). T2-DM rats were defined as animals with a fasting blood glucose level of greater than 250 mg/dl.

Experimental protocol

Thirty male Sprague Dawley rats were split into five groups (n = 6) as follows; Gp1 was a negative

control. Gp2 was served as diabetic rats only. Gp3, Gp4, and Gp5 received Met (150 mg/kg b.wt), RNV (7.5 mg /kg b.wt), and RNUE (14.4 mg /kg b.wt), respectively. All treatments were taken i.p., once every day for eight weeks. At the end of the experiment, rats were given 2% isoflurane anesthesia before necropsy.

Determination of the total body weight changes

All the experimental groups were weighted at the beginning of the experiment at week 0 (WK-0). Normal and HFD-rats were weighted again at WK-12 (before treatment) and Wk-20 as initial (I.b.wt) and final body weights (F.b.wt), respectively.

Determination of the biochemical parameters

Sera glucose and C-peptide levels were determined in all groups (30,31). Liver aminotransferase (ALT and AST) activities were determined according to the method of (32). Urea and creatinine levels were determined according to (33). Superoxide dismutase (SOD), catalase (CAT) activities, and malondialdehyde (MDA) levels were determined according to (34-36).

Histopathological investigations

Small sections of the pancreas were fixed in 10% neutral formalin, dehydrated in alcohols, cleared in xylene, and embedded in paraffin. Hematoxylin and eosin (H& E) staining was used on paraffin slices that were 5 mm thick (37).

Immunohistochemical staining and image analysis:

Detection of anti-insulin monoclonal antibodies in pancreatic tissues was performed using the avidin-biotin-peroxidase method (38). The insulin-secreting β-cells cytoplasmic sites of reaction were stained brown, and nuclei stained blue. Digital images were analyzed by a semi–quantitative scoring system (Figi–image J software, a Java-based application for analyzing images). The positive stained cells were analyzed in pancreas sections. The percentage-colored stained area (area fraction) per field area was determined by measuring 30 randomly photographed high-power fields at x 40 magnifications from islets of Langerhans (39).

Statistical analysis

Group's data expressed as means ± S.D. were analyzed by t-test while percentage data were analyzed by SPSS software. $p < 0.05$ was considered a significant value for all statistical analyses in this study.

RESULTS

Scyphozoan specimen morphometric characterization

The scyphozoan R. nomadica was identified from the collected specimens. The specimens have a hemispherical bowl-shaped umbrella with no marginal tentacles and are divided into two parts. The white-colored umbrella is thick at the central ex-umbrella and thinning gradually towards the marginal sub-umbrella. *R. nomadica* is characterized by the presence of obvious minute rough granules on ex-umbrella, and few at the margin. The margin is divided into 64 rounded velar lappets. The scapulats are followed by eight adradial stout and smooth icy, blue-colored oral arms that seem to be branched, broad, and polystomatous **(Figure 1)**. The umbrella diameters of *R. nomadica* ranged between 35- 40 cm, and the weight of the specimens ranged between 4- 4.5 kg**.**

Biochemical measurements and protein profile of RNV and RNUE

The macromolecules as total protein, lipid, and carbohydrate of RNV were 380 ± 22, 122 ± 11.9, and 44 ± 5.7 mg/g of dry weight (D.W), respectively. These macromolecules in RNUE were 240 ± 18.5, 102.6 ± 10.3 and 25.6 ± 4.8 mg/g D.W, respectively (Table 1). The protein profile of RNV showed eight bands from 10 to 50 kDa molecular weight (Mwt). At the same time, RNUE showed seven bands with Mwt from 40 to 100 kDa. **(Figure 2A and B).**

The DPPH scavenging activity percentage, IC_{50} of DPPH, and the total antioxidant capacity (TAC) in RNV were 7.03 %, 698.56 mg/ml, and 2.39 ± 0.18 mg/g. D.W, respectively. These biochemical parameters in RNUE were 9.67 %, 506.71 mg/ml, and 2.92 ± 0.26 mg/g. D.W **(Table 1).**

The LD_{50} values of RNV and RNUE

Different groups of rats were injected i.p. once with different doses of RNV or RNUE. The groups were monitored for 24 hours to determine the mortality rates. The results showed that the LD_{50} of RNV and RNUE were 310.72 and 576.9 mg/kg b.wt, respectively **(Figure 3 A, B).**

Treatment of T2-DM rats with RNUE potentially decreased the body weight.

The T2-DM rats showed a slight change in the b.wt, however, treatment of T2-DM groups with Met or with RNUE showed a highly significant decrease ($p < 0.01$) in the b.wt when compared to the T2-DM group. Additionally, T2-DM rats treated with RNV led to a significant decrease ($p < 0.05$) in b.wt as compared to T2-DM rats **(Figure 4).**

RNUE dramatically decreased glucose and C-peptide levels in T2-DM rats

The T2-DM rats that were treated with Met or RNUE showed a significant decrease ($p < 0.05$) in glucose and C-peptide levels, however, T2-DM rats that were treated with RVE showed a significant increase ($p < 0.05$) in the levels of glucose and C-peptide **(Figure 5A, B).**

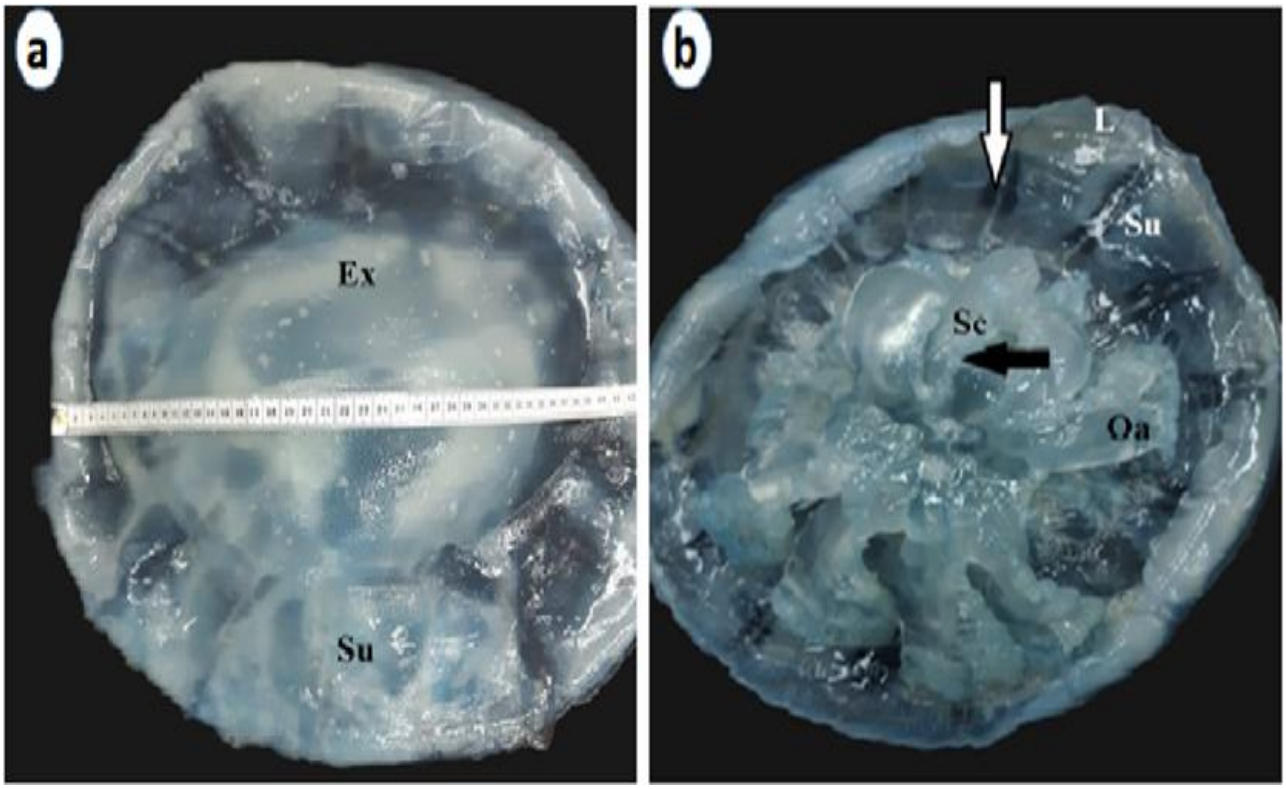

Figure 1. Shows the exumbrellar and subumbreller side of *R. nomadica.* (**a**) Exumbrellar side showing thick, rough central granulated exumbrella (Ex), thin subumbrella (Su) end with marginal lappet (L). (**b**) Subumbreller side showing canal system (white arrow), and at the base of the oral cone, the scapulat (Sc) with frilly mouths (black arrow) and wide polystomatous oral arms (Oa).

Table 1. The total protein, lipids, carbohydrates contents, the DPPH scavenging activity %, IC_{50} of DPPH, and the total antioxidant capacity of RNV and RNUE.

	Total proteins (mg/g DW)	Total lipids (mg/g DW)	Total carbohydrates (mg/g DW)	DPPH scavenging activity (%)	IC_{50} of DPPH (mg/ml)	TAC (mg/g D.W)
RNV	380 ± 22 [a]	122 ± 11.9 [a]	44 ± 5.7 [a]	7.03[a]	698.56[a]	2.39 ± 0.18[a]
RNUE	240 ± 18.5 [b]	102.6 ± 10.3 [b]	25.6 ± 4.8 [b]	9.67[b]	506.71[b]	2.92 ± 0.26[a]

RNV: *R. nomadica* venom; **RNUE:** *R. nomadica* umbrella extract; **TAC:** Total antioxidant capacity. *p*-value < 0.05 was considered to be statistically significant. The means that do not share the same letter are significantly different.

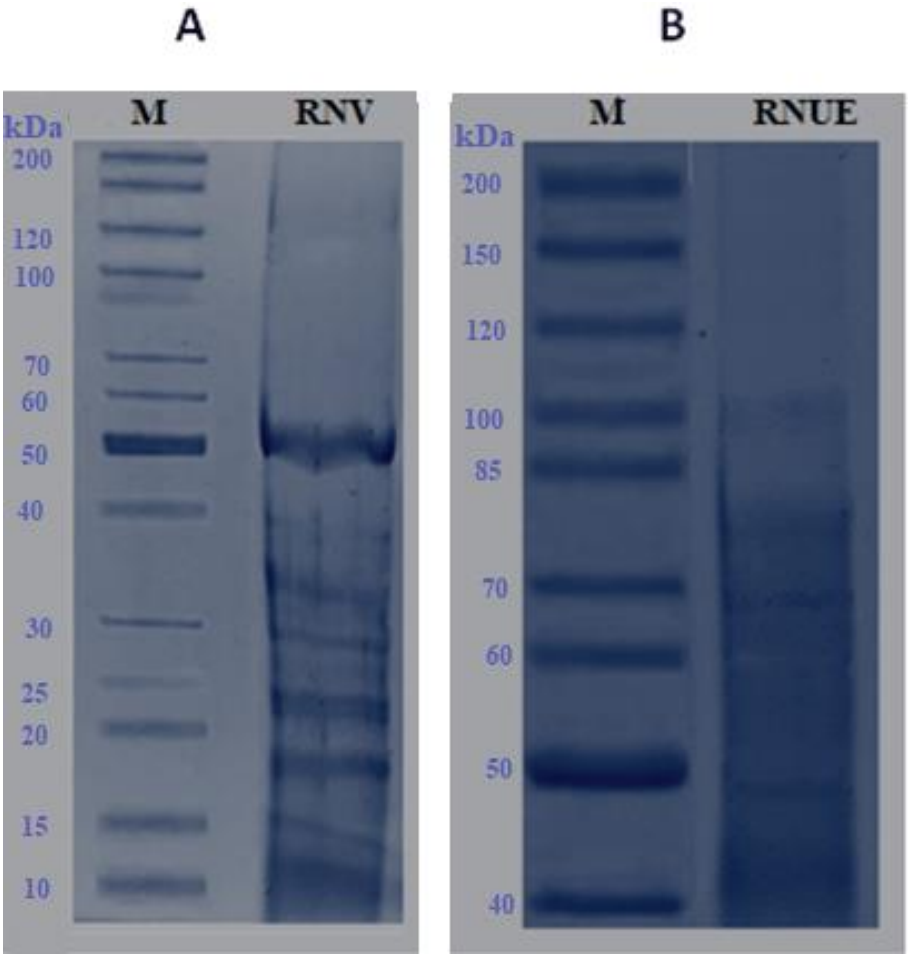

Figure 2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) of *R. nomadica* venom **(A)** and umbrella extract **(B)**. M: protein standard marker 10-200 KDa.

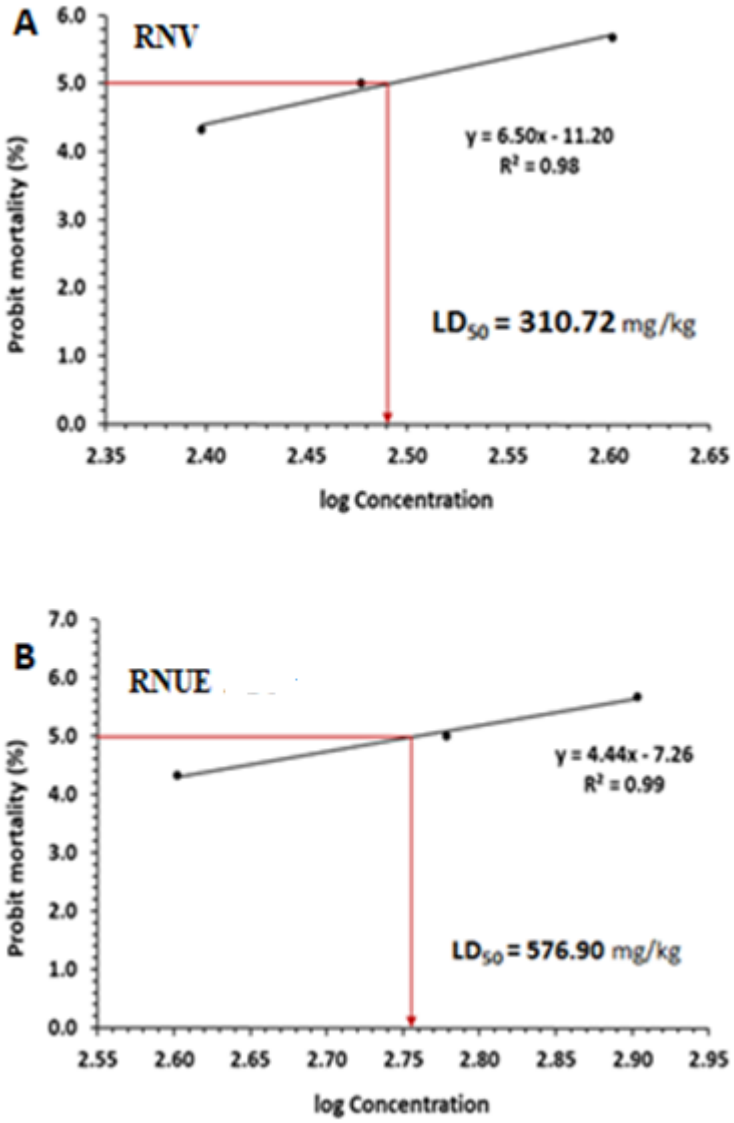

Figure 3. Shows the medial lethal doses (LD_{50}) of *R. nomadica* venom (RNV) (A) and *R. nomadica* umbrella extract (RNUE) (B).

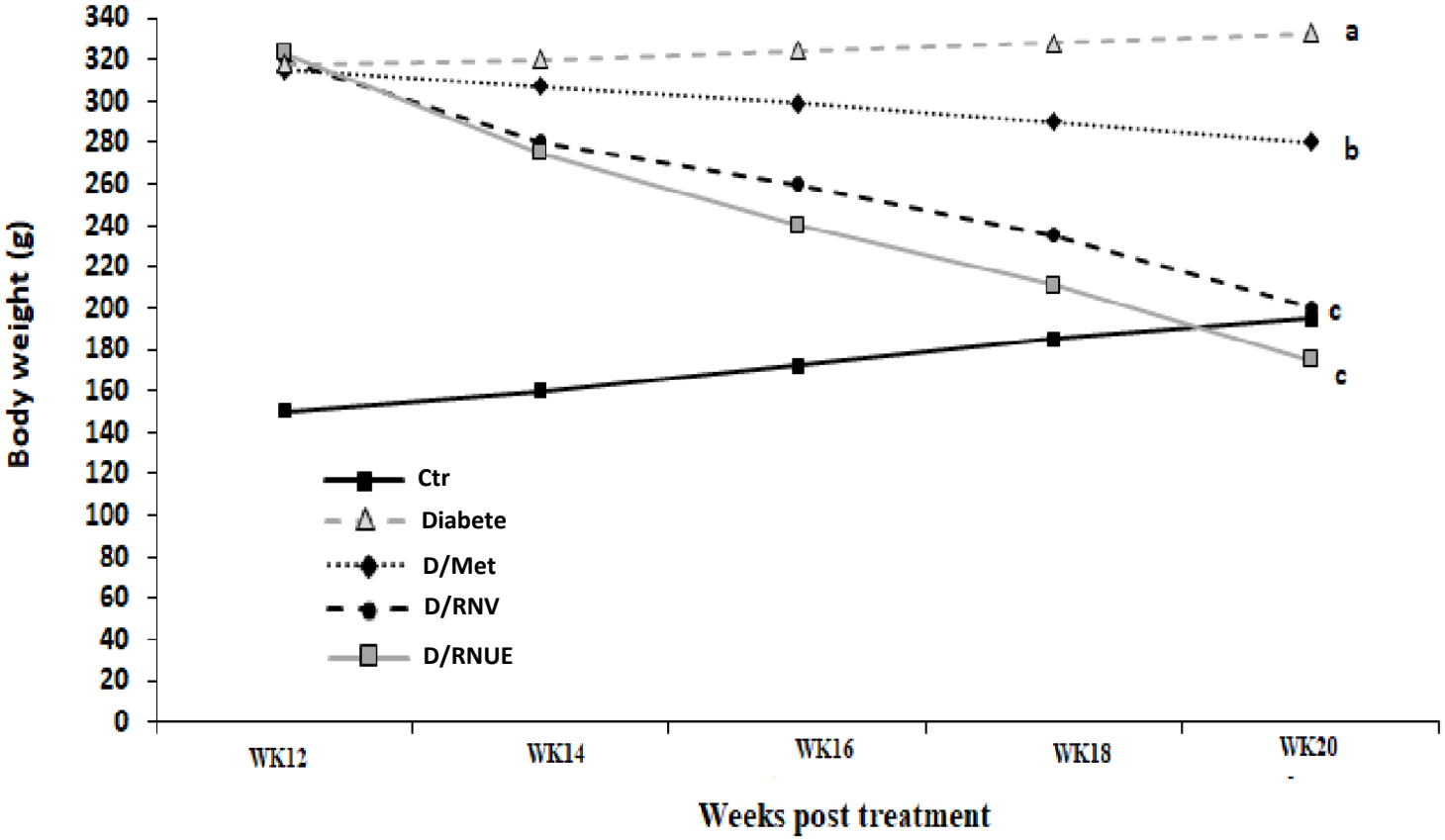

Figure 4. The kinetic changes in the total body weight of the different treated groups from wk 12 (Before-treatment) until wk 20 (post-treatment). **Ctrl:** Control group; **RNE:** *R. nomadica* venom; **RNUE:** *R. nomadica* umbrella extract; **D:** Diabetic group. The values represented mean ± SD. *p*-value < 0.05 was considered to be statistically significant. The means that do not share the same letter are significantly different.

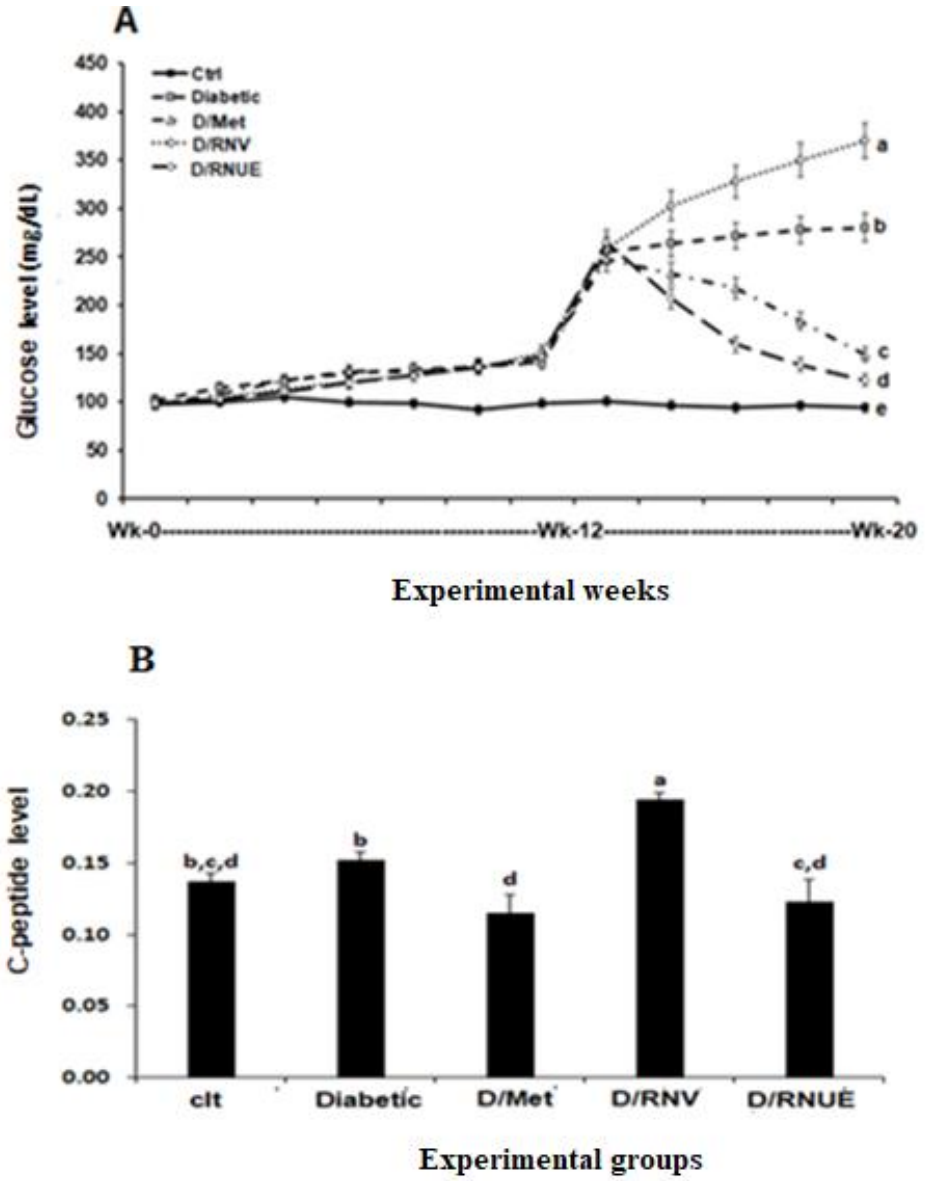

Figure 5. Shows the kinetic changes in the glucose levels (A), the glucose levels (B), and the C-peptide levels in different groups. **Ctrl:** Control group; **RNE:** *R. nomadica* venom; **RNUE:** *R. nomadica* umbrella extract; **D:** Diabetic group. The values represented mean ± SD. *p*-value < 0.05 was considered to be statistically significant. The means that do not share the same letter are significantly different.

RNUE treatment improved the liver transaminases and kidney biomarkers in T2-DM rats:

The T2-DM rats showed a significant increase ($p < 0.05$) in liver transaminase (ALT and AST) activities. T2-DM rats that were treated with Met or RNUE showed a significant decrease ($p < 0.05$) in ALT and AST activities compared to T2-DM rats. However, T2-DM rats that were treated with RNV showed a significant decrease in the above-mentioned liver transaminase activities ($p < 0.05$) when compared to T2-DM rats **(Table 2)**. Similarly, the results that were obtained from kidney biomarker analysis showed that, as compared to the control group, the levels of urea and creatinine had a significant increase ($p < 0.05$) in the T2-DM rats. T2-DM rats that were treated with Met or RNUE showed a significant decrease ($p < 0.05$) in the levels of urea and creatinine when compared with T2-DM rats. However, T2-DM rats that were treated with RNV showed a significant increase in the above-mentioned parameters ($p < 0.05$) when compared to T2-DM rats **(Table 2)**.

Treatment with RNV or RNUE enhanced the oxidant/antioxidant status in the T2-DM rats:

The T2-DM rats showed a significant decrease ($p < 0.05$) in the antioxidant enzymes (SOD and CAT) activities as compared to the control group. T2-DM rats that were treated with Met or RNUE showed a significant increase ($p < 0.05$) in SOD and CAT activities compared to T2-DM rats. However, T2-DM rats that were treated with RNV showed a significant decrease in the above-mentioned antioxidant enzymes ($p < 0.05$) when compared to T2-DM rats **(Table 3).** The level of MDA had a significant increase ($p < 0.05$) in the T2-DM rats. T2-DM rats that were treated with Met or RNUE showed a significant decrease ($p < 0.05$) in the level of MDA, however, T2-DM rats that were treated with RNV showed a significant increase in MDA level ($p < 0.05$) when compared to T2-DM rats **(Table 3).**

RNUE enhanced the pancreatic architecture in T2-DM rats:

The examination of H&E-stained pancreatic sections of the control group showed normal architecture of the pancreatic acini and islet of Langerhans. As shown in **Figure 6A**, the pancreatic acini are made up of pyramidal cells with basal rounded nuclei, and the cytoplasm of each pyramidal cell includes acidophilic zymogen granules. The connective tissue is very little between two acini and the islets of Langerhans appear as non-capsulated pale-stained rounded or oval areas inside the pancreatic lobules. However, the pancreatic sections of the T2-DM rats showed destruction of islet cells with an irregular shape, the presence of necrotic areas, islet cells vacuolation with hypertrophied nuclei, and atrophied cytoplasm. Also, the disorganization of acinar cells periphery to the condensed fibers around the intercalated duct, with infiltration of the inflammatory leucocytes was noticed **(Figure 6B)**. In T2-DM rats that were treated with Met, the pancreatic sections showed normal of most islet cells with well-outlined boundaries, minimization of the vacuolated islet cells, and appearance of normal acinar cells **(Figure 6C).** The pancreatic tissue of the T2-DM rats treated with RNV showed severe degenerative changes in the pancreatic islets and exocrine pancreas with necrotic areas. Some acinar cells had become enlarged with foamy or completely vacuolated cytoplasm. Infiltration of the inflammatory leucocytes, intense fibrosis around many congested blood vessels, and dilated ducts were seen **(Figure 6D).**

Examination of the pancreatic section of T2-DM rats treated with RNUE revealed a marked improvement of pancreatic islet and acinar cells to normal appearance. The vacuolation was also reduced or absent in many islets, and normal distribution of fibers around blood vessels was detected **(Figure 6E).**

Treatment with RNUE restored insulin-secreting β-cells in T2-DM rats:

The IHC examination showed strong positive immuno-stain expression of the insulin-secreting β-cells in the central zone of the islets **(Figure 7A)**. The pancreatic sections of T2-DM rats showed an obvious decline in immunoreaction with less expression of insulin-secreting β-cells **(Figure 7B).** However, the T2-DM rats treated with Met showed an increase in the expression of insulin-secreting β-cells **(Figure 7C).** A significant decrease in the expression of the insulin-secreting β-cells (weak positive) was reported in the pancreatic tissues of T2-DM rats that were treated with RNV **(Figure 7D)**. Interestingly, an obvious recovery of insulin-secreting β-cells (strongly positive) was noticed in the pancreatic sections of T2-DM rats that were treated with RNUE **(Figure 7E).** The mean values of percentage area ± SD of insulin reactive β-cells are significantly decreased ($p < 0.001$) in both T2-DM rats and T2-DM rats that were treated with RNV in comparison with control rats. While the T2-DM rats that were treated with RNUE recorded a major proliferation in the area of insulin reactive β-cells **(Table 4).**

Table 2. ALT, AST activities, urea, and creatinine levels in different experimental groups

Groups	ALT (U/L)	AST (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
Ctrl	40.60±2.16 ^{c,e}	70.10±10.55 ^{b}	42.83 ± 4.26^{c}	0.73 ± 0.02^{b}
Diabetic	68.81±2.99 ^{b}	120.80±11.90 ^{a}	67.75 ± 5.56^{a}	0.87 ± 0.02^{a}
D/Met	51.25±5.07 ^{d}	95.65±13.30 ^{d}	57.93 ± 2.17^{d}	0.74 ± 0.06^{b}
D/RNV	85.87±7.51 ^{a}	146.73±5.51^{e}	73.67 ± 3.06^{b}	0.95 ± 0.09^{e}
D/RNUE	46.17±2.52 ^{d,e}	85.20±3.61$^{c, d}$	51.67 ± 1.53c$^{, d}$	0.74 ± 0.03^{b}
F-Value	**231.39**	**83.89**	**215.27**	**14.22**
P-Value	**<0.001**	**<0.001**	**<0.001**	**<0.001**

ALT: Alanine aminotransferase; **AST:** Aspartate aminotransferase. *p*-value < 0.05 was statistically significant. **Ctrl:** Control group; **D/Met**: diabetic rats that treated with metformin, **D/RNV:** diabetic rats that treated with *R. nomadica* venom; **D/RNUE:** diabetic rats that treated with *R. nomadica* umbrella extract. The values represented mean ± SD. The means that do not share the same letter are significantly different.

Table 3. Antioxidant/ oxidant biomarkers in different experimental groups

Groups	SOD (U/mg tissue)	CAT (U/mg tissue)	MDA (nmol/mg tissue)
Ctrl	14.89 ± 0.74 ^{a}	82.86 ± 1.16 ^{a}	34.29 ± 1.34 ^{e}
Diabetic	5.85 ± 1.07 ^{d}	51.04 ± 2.13 ^{d}	81.91 ± 2.16 ^{b}
D/Met	8.51 ± 0.82 ^{c}	60.85 ± 2.70 ^{b}	59.92 ± 1.98 ^{d}
D/ RNV	3.2 ± 0.40 ^{e}	42.24 ± 1.55 ^{e}	108.21 ± 2.13 ^{a}
D/RNUE	11.16 ± 1.06 ^{b}	76.91 ± 1.41 ^{c}	46.89 ± 1.87 ^{c}
F-Value	15.34	185.4	567.0
P-Value	<0.001	<0.001	<0.001

Ctrl: Control group; **D/Met**: diabetic rats that were treated with metformin, **D/RNV:** diabetic rats that were treated with *Rhopilema nomadica* venom; **D/RNUE:** diabetic rats that were treated with *R. nomadica* umbrella extract, **SOD:** Superoxide dismutase; **CAT:** Catalase; **MDA:** Malondialdehyde. The means that do not share the same letter are significantly different. The values represented mean ± SD. *p*-value < 0.05 was statistically significant.

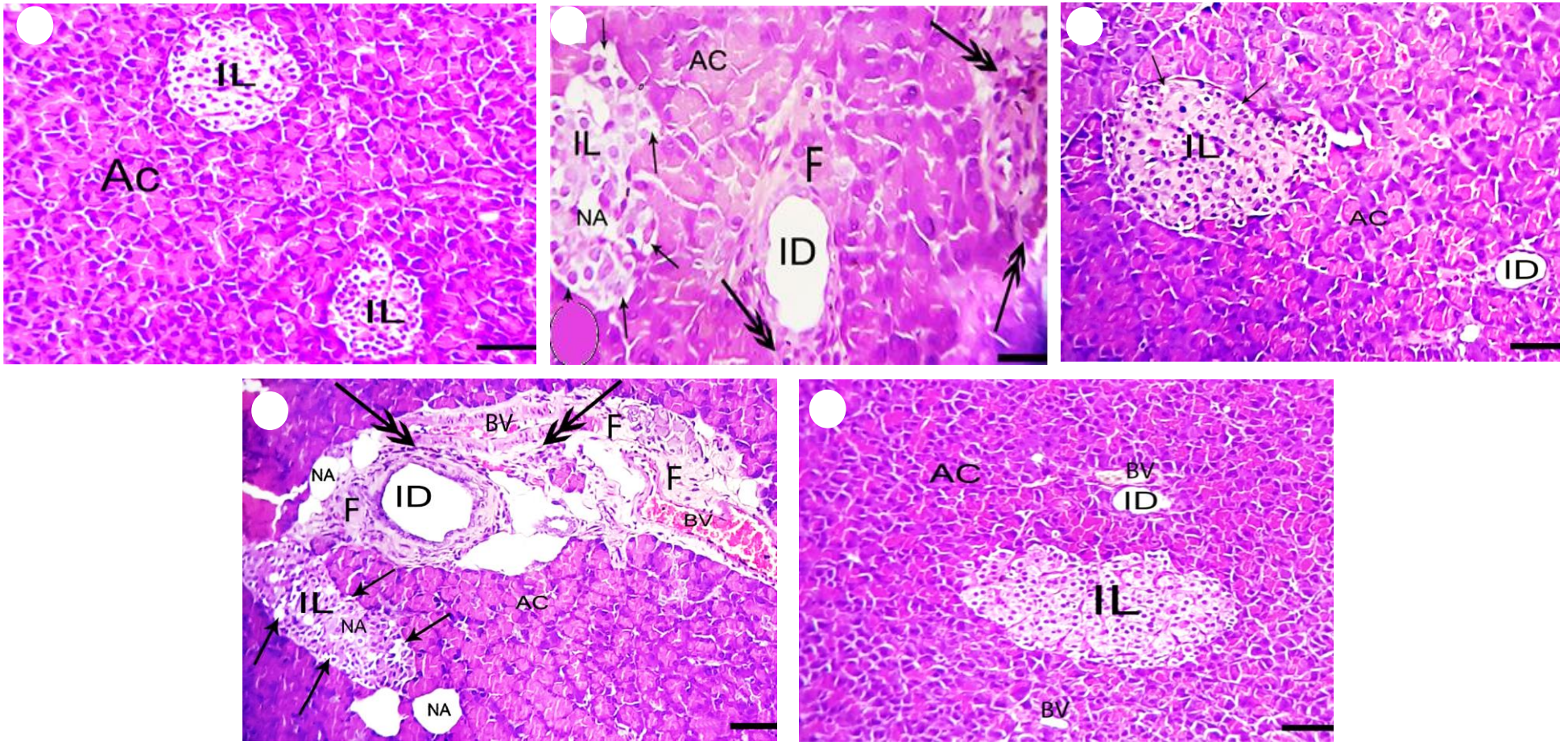

Figure 6. (**A**) Photomicrograph of a pancreatic section of a normal control rat showing normal architecture of acini (AC) and islets of Langerhans (IL). (**B**) Photomicrograph of pancreatic section of T2-DM rats illustrating necrotic areas (NA), vacuolated cells (arrows) in the islets of Langerhans (IL), disorganization of acinar cells (AC), condensed fibers (F) around intercalated duct (ID) and infiltration of the inflammatory leucocytes (double- arrowheads) is also seen. (**C**) Photomicrograph of a pancreatic section of T2-DM rats treated with Met showing the minimization of the vacuolated islet cells (arrows) with the appearance of normal of most islet cells (IL), The normal form of acinar cells (AC) and intercalated duct (ID) lined with more or less normal cuboidal cells are also seen. (**D**) Photomicrograph of a pancreatic section of T2-DM rats treated with RNV demonstrating cytoplasmic vacuolation in most of the islet cells (IL) (arrows), increasing the necrotic areas (NA) in both IL& AC, anomalous acinar cells (AC), markedly accumulated fibers (F) around dilated intercalated duct (ID) and congested blood vessels (BV). Infiltration of inflammatory leucocytes is seen (double-arrowheads). (**E**) Photomicrograph of pancreatic section of T2-DM rats treated with RNUE dedicating a marked improvement of pancreatic islet (IL) and acinar cells (AC). Recovery of the normal blood vessels (BV) and intercalated duct (ID) are also demonstrated (E). H&E, scale bar = 6.25µm.

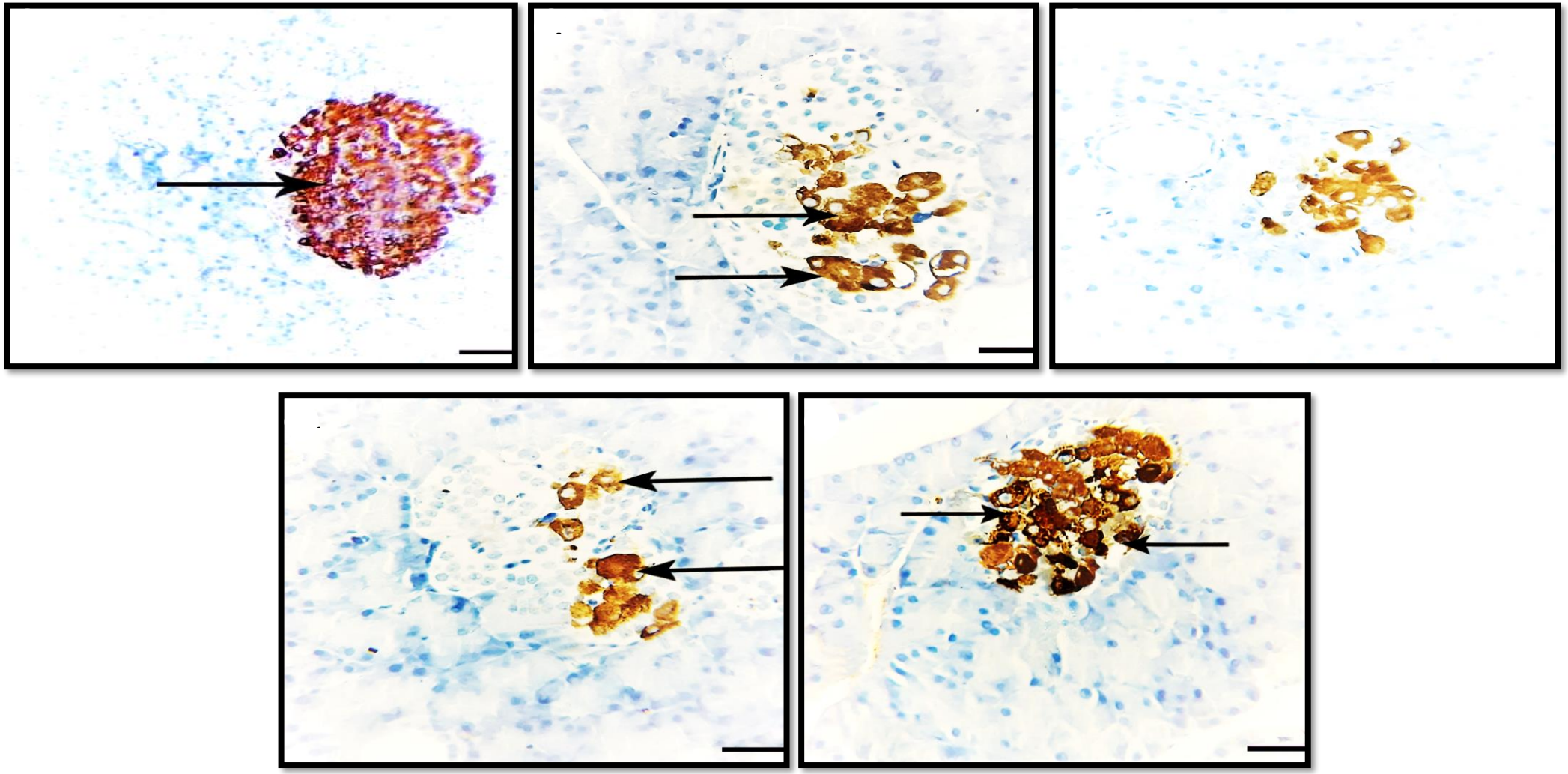

Figure 7. (A) Photomicrograph of pancreatic sections of control rats showing strong expression of insulin-secreting β-cells (arrows). (**B**) Photomicrograph of pancreatic section of STZ-diabetic rats demonstrating a decrease in immunostain expression of insulin-secreting β-cells (arrows). (**C**) Photomicrograph of pancreatic section of T2-DM rats treated with Met showing an increase in the expression of insulin-secreting β-cells. (**D**) Photomicrograph of pancreatic section of T2-DM rats treated with RNV demonstrating a severe reduction of immunostain expression of the insulin-secreting β-cells (arrows). (**E**) Photomicrographs of pancreatic sections of T2-DM rats treated with RNUE show a marked increase and recovery in the expression of immunoreactivity to insulin-secreting β-cells (arrows) (E). Scale bar = 6.25 µm.

Table 4. The percentage of the area of the positive insulin-secreting β-cell in the pancreas of different groups.

Groups	Insulin
Control	4.91 ± 0.33 [a]
Diabetic	1.75 ± 0.15 [c]
D/Met	2.84 ± 0.12[e]
D/RNV	0.74 ± 0.08 [d]
D/RNUE	3.72 ± 0.13 [b]
F-Value	**92.84**
P-Value	**< 0.001**

Ctrl: Control group; **D/Met**: diabetic rats that treated with metformin, **D/RNV:** diabetic rats that treated with *R. nomadica* venom; **D/RNUE:** diabetic rats that treated with *R. nomadica* umbrella extract. The values represented mean ± SE. *p*-value < 0.05 is statistically significant. The means that do not share the same letter are significantly different.

Discussion

Diabetes mellitus (DM) has emerged as one of the most frequent major chronic illnesses, generating life-threatening complications and diminishing life expectancy. As a result, ongoing research has been conducted to identify novel antidiabetic drugs with high efficacy and minimal side effects for managing and treating diabetes (40). According to the current study, the diameters and weights of the collected *R. nomadica* were 35- 40 cm and 4-4.5 kg. According to prior research, its umbrella diameter was 38 cm while its weight was 4.2 kg (41). The LD50 values for RNV and RNUE were 0.311 and 0.577 g/kg b.wt, respectively. Previous studies showed that the LD_{50} values of *R. octopus* venom and *Aurelia aurita* in mice were 1.8 and 2 g/kg b.wt, respectively (10,42). DM rats showed weight loss compared to control rats. D/Met, D/RNV, and D/RNUE rats lost weight compared to DM rats. Previous studies showed that significant change in the body weight in T2-DM rats, and this change increased by Met treatment (43). A significant change in the glucose and C-peptide levels was reported in the T2-DM group. This data was covenant with a prior study that reported that T2-DM increased both glucose and C-peptide levels (44). T2-DM rats that were treated with Met showed also a significant decrease in the previous parameters, and these findings were in agreement with Ibrahim et al, 2023 who reported that Met decreased glucose and C-peptide levels (45). T2-DM rats treated with RNV showed non-significant changes in the levels of glucose and C-peptide, while T2-DM rats that were treated with RNUE did show a significant decrease in their levels. Talluri et al, 2018 evaluated the methanolic extract of *A. aurita* as an anti-diabetic agent (42).

The current study showed that the T2-DM group did show a significant increase in ALT and AST activities. Talluri et al, 2018 revealed that the STZ-induced T2-DM rats showed elevation in AST, and ALT activities (42). T2-DM rats that were treated with Met did show a significant decrease in ALT and AST activities (46). This study showed that T2-DM rats that were treated with RNV showed non-significant changes in the activities of ALT and AST, while the T2-DM group that was treated with RNUE did show a significant decrease in their activities. The decrease in AST and ALT activities could be due to the potential hepatoprotective properties of RNUE.

In this study, the T2-DM group did show a significant decrease in SOD and CAT activities. This decrease could be due to the fluctuations in blood glucose levels which in turn led to elevation in reactive oxygen species (ROS) which consequently decreased the activities of these enzymes (46). Treatment with Met, however, showed a significant increase in these antioxidant enzymes. Met has been reported to have antioxidant activity against oxidative damage caused by ROS (47). T2-DM rats that were treated with RNV showed non-significant changes in SOD and CAT activities. In contrast, RNUE treatment did show a significant increase. The MDA level increased in T2-DM rats (46). Treatment with Met or RNUE decreased the MDA level, while RNV increased this level. The enhancement in the antioxidant/ oxidant biomarkers status could be due to the presence of bioactive ingredients in RNUE. Similarly, and consistent with this hypothesis, a previous study showed that the proteins isolated from both Asiatic and Mediterranean jellyfish have a strong radical scavenging activity and reducing power (48). Additionally, proteins could own antioxidant properties which could inhibit lipid oxidation through multiple pathways including inactivation of ROS, scavenging free radicals, and chelation of prooxidative transition metals (49). Several researchers reported the antioxidant activities of edible jellyfish (48,50-51).

Streptozoticin causes a selective loss of the insulin-secreting pancreatic β-cells via ROS-dependent oxidative damage which in turn leads to inhibition of insulin secretion of β cells, and disruption of glucose metabolism (52). The pancreatic tissues of the T2-DM rats displayed degenerative and necrotic changes in pancreatic β cells. Also, dilation of the intercalated duct and infiltration of inflammatory leucocyte cells. These changes in pancreatic architectures are in agreement with Dludla et al, 2023 (53).

D/Met or D/RNUE-treated rats demonstrated reduced and better pancreatic tissue alterations, as well as increased expression of insulin-secreting -cells in the islets of Langerhans. Met therapy improved the histological alterations in pancreatic islets in a moderate but substantial way. Similarly, after Met therapy, islet insulin content increased considerably. Met increases insulin binding to insulin receptors, lowering blood glucose levels, indicating that pancreatic -cells are not damaged in T2-DM. The level of insulin antibody shows signs of reduction of STZ-induced -cell toxicity following Met treatment (54). D/RNV demonstrated substantial degenerative alterations in the pancreatic islets and exocrine pancreas, as well as necrotic regions. It is worth mentioning that jellyfish is enriched in collagenous protein, which is supposed to have higher antioxidant, and anti-inflammatory activities, hyperglycemia, and hyperlipidemia (55).

CONCLUSION

This study shed light on the potential anti-diabetic effect of the RNUE and its ameliorative effect on the pancreatic architecture of diabetic rats.

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REFERENCES

1. Li, G., Chen, Z. Lv, Z., Li, H., Chang, D., & Lu Jinping, (2021). Diabetes Mellitus and COVID-19: Associations and Possible Mechanisms. International Journal of Endocrinology, Volume, Article ID 7394378, 10 pages.
2. Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., Stein, C., Basit, A., Chan, C.N, Mbanya, J. C., Pavkov, M. E., Ramachandaran, A., Wild, S. H., James, S., Herman, W. H., Zhang, P., Bommer, C., Kuo, S., Boyko, E.J.& Magliano, D. J. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes research and clinical practice, 183, 1091193.

3. Magliano, D.J., Islam, R.M., Barr, E.L.M., Gregg, E.W., Pavkov, M.E., & Harding, J.L. (2019). Trends in incidence of total or type 2 diabetes: systematic review**.** British Medical Journal,366,15003
4. Boyle, P., Boniol, M., Koechlin, A., Robertson, C., Valentini, F., & Coppens, K. (2012). Diabetes and breast cancer risk: a meta-analysis. British Journal of Cancer,107,1608-17
5. Lester, F.T. (2019). Diabetes mellitus. In: The ecology of health and disease in Ethiopia. Routledge, London.
6. Blahova, J., Martiniakova, M., Babikova, M., Kovacova, V., Mondockova, V., & Omelka, R. (2021). Pharmaceutical drugs and natural therapeutic products for the treatment of type 2 diabetes mellitus. Pharmaceutical, 14, 806.
7. Davies, M.J., Aroda, V.R., Collins, B.S., Gabbay, R.A., Green, J., Maruthur, N.M., Rosas, S.E., Del Prato, S., Mathieu, C., Mingrone, G., Rossing, P., Tankova, T., Tsapas, A., & Buse, J.B. (2022). Management of Hyperglycemia in Type 2 Diabetes, 2022. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care, 45(11), 2753-2786. doi: 10.2337/dci22-0034. PMID: 36148880; PMCID: PMC10008140.
8. Elbakry, M. & Elremaly, W. (2023). Antidiabetic activity of some common medicinal plants. Biological and Biomedical Journal, 1(2), 1-16. doi: 10.21608/bbj.2023.321880.
9. Tariq, R., Khan, K., Ali, R., & NaeemWain, Z. (2016). Natural remedies for diabetes mellitus. International. Current. Pharmacology Journal, 5, 97.
10. Salama, W.M, El-Naggar, S.A, Attia, H.A., & Hamed, E.H. (2022). The cytotoxic effect of rhizostome jellyfish venom on cancer cell lines and its antitumor activity in ehrlich ascites-bearing mice. Egyptian Journal of Zoology (EJZ),78,15-30
11. Molinski, T.F., Dalisay, D.S., Lievens, S.L., & Saludes, J.P. (2009). Drug development from marine natural products. Nature Reviews Drug Discovery, 8: 69-85
12. D'Ambra, I.& Lauritano, C. (2020). A Review of toxins from Cnidarian. Marine Drugs, 18, 507.
13. Hsieh, Y.H.P., Leong, F.M., & Rudloe, J. (2001). Jellyfish as food. Hydrobiologia, 451,11-17.
14. Madkour, F. F., Safwat, W., & Hanafy, M. H. (2019). Record of aggregation of alien tropical schyphozoan Rhopilema nomadica Galil, 1990 in the Mediterranean Coast of Egypt. International Mad Studies Journal (IMSJ), 1(2), 1-7.
15. Ding, J. F., Li, Y. Y., Xu, J. J. *et al*. (2011). Study on effect of jellyfish collagen hydrolysate on anti-fatigue and anti-oxidation. Food Hydrocolloids, 25(5), 1350-1353.
16. Brinkman, D. L., Konstantakopoulos, N., Mclnerney, B. V. *et al*. (2014). Chironex fleckeri (box jellyfish) venom proteins: expansion of a cnidarian toxin family that elicits variable cytolytic and cardiovascular effects. Journal of Biological Chemistry, 289(8), 4798- 4812.
17. Frazao, B. & Antunes, A. (2016). Jellyfish bioactive compounds: methods for wet-lab work. Marine Drugs, 14(4), 75. DOI: 10.3390/md14040075.
18. Salemi, Z., Rafie, E., Goodarzi, M.T., & Ghaffari, M.A. (2016): Effect of Metformin, acarbose and their combination on the serum level in nicotinamide/streptozocin-induced type 2 diabetic rats. Iranian Red Crescent Medical Journal, 18(3), 23814.

19. Jouiaei, M., Casewell, N.R., Yanagihara, A.A., Nouwens, A., Cribb, B.W., Whitehead, D., Jackson, T.N., Ali, S.A., Wagstaff, S.C., Koludarov, I., Alewood, P., Hansen, J., & Fry, B.G. (2015). Firing the sting: chemically induced discharge of cnidae reveals novel proteins and peptides from box jellyfish (*Chironex fleckeri*). Venoms and. Toxins Journal**,** 7(3), 936-950.
20. Yanagihara, A., Kuroiwa, J., Oliver, L., Chung, J., Kunkel, D.D. (2002). Ultra structure of a novel eurytele nematocyst of *Carybdea alata* (Cubozoa: Cnidaria). Cell Tissue Research, 308, 307-318.
21. Stoscheck, C. M. (1990). Quantitation of protein. Methods of Enzymology, 182, 50-68.
22. Folch, J., Lees, M. & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226, 497- 509.
23. Plummer, D. T. (2006). An Introduction to Practical Biochemistry (3rd Edition). Tata McGraw Hill Publishing Company, Noida, Uttar Pradesh, India.
24. Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 22, 680-685.
25. Brand-Williams, W., Cuvelier, M.E., & Berset, C.L. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology, 28(1), 25-30.
26. Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical biochemistry, 269(2), 337- 41.
27. Finney, D. J. & Stevens, W. L. (1948). A table for the calculation of working probits and weights in probit analysis. Biometrika, 35(1-2), 191-201.
28. Levin, B. E. & Dunn-Meynell, A. A. (2002). "Defense of body weight depends on dietary composition and palatability in rats with diet-induced obesity," The American Journal of Physiology— Regulatory Integrative and Comparative Physiology, 282(1), 46–54.
29. Xiang, X., Wang, Z., Zhu, Y., Bian, L., Yang Y. (2010). [Dosage of streptozocin in inducing rat model of type 2 diabetes mellitus]. Wei Sheng Yan Jiu. Mar;39(2), 138-42. Chinese. PubMed (MID): 20459021
30. Tietz, N.W., ed. (1995): Clinical guide to laboratory tests. 3rd ed . Philadelphia: WB Saunders. 268-273.
31. Jones, A.G., & Hattersley, A.T. (2013). The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabetic Medicine, 30, 803-817.
32. Rei, R. (1984). Measurement of aminotransferase: Part I. Aspartate aminotransferase. Critical Reviews in Clinical Laboratory Sciences, 21, 99-186.
33. Thomas, L. (1998). Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaf, 27, 644-647.
34. Nishikimi, M., Rao N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research Communications, 46, 849-853.
35. Aebi, H. (1984). Catalase *in vitro*. Methods Enzymology, 105, 121-126.
36. Li, X.Y., & Chow, C.K. (1994). An improved method for the measurement of malondialdehyde in biological samples. Lipids, 29, 73-75.

37. Bancroft, J.D. (2008). Theory and practice of histological techniques 6th ed. Churchill Livingstone, Elsevier Philadelphia, PA: Elsevier, H Sci.
38. Hsu, S.M., Raine, L., & Fanger, H. (1981). Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. Journal of. Histochemistry &Cytochemistry, 29(4), 577-580.
39. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: an open source platform for biological-image analysis. Nature Methods, 9 (7), 676-682.
40. Frier, B.M. (2014). Hypoglycaemia in diabetes mellitus: epidemiology and clinical implications. Nature Reviews Endocrinology,10 (12),711-22.
41. Gülşahin, N. & Tarkan, A.N. (2011). The first confirmed record of the alien jellyfish *Rhopilema nomadica* Galil, 1990 from the southern Aegean coast of Turkey. Aquatic Invasions, 6, S95-S97
42. Talluri, M. R., Ketha, A., & Battu, G. R. (2018). Protective effect of *Aurelia aurita* against free radicals and streptozotocin-induced diabetes. Bangladesh Journal of Pharmacology. 13, 287-295.
43. Al Zaabi, M., Ali, B. H., Al Suleimani, Y., Adham, S.A., Ali, H., Manoj, P., Ashique, M., & Nemmar, A. (2021). The Effect of Metformin in Diabetic and Non-Diabetic Rats with Experimentally-Induced Chronic Kidney Disease. Biomolecules,11(6),81.
44. Qinna, N.A., & Badwan, A.A. (2015). Impact of streptozotocin on altering normal glucose homeostasis during insulin testing in diabetic rats compared to normoglycemic rats. Drug Design Development and Therapy, 9, 2515-25.
45. Ibrahim, Y., Fatma, F., Elshymaa, A., & Zahra, S. (2023). Pathophysiological mechanisms of type 2 diabetes mellitus and the effects of metformin treatment in adult male albino rats. Minia Journal of Medical Research (MJMR), 34, 209-214.
46. Sani, M., Yaghmaei P., Hajebrahimi Z., & Roodbari N.H. (2022). Therapeutic Effect of P-Cymene on Lipid Profile, Liver Enzyme, and Akt/Mtor Pathway in Streptozotocin-Induced Diabetes Mellitus in Wistar Rats. Journal of Obesity, 1-12.
47. Cahova, M., Palenickova, E., Dankova, H., Sticova, E., Burian, M., Drahota, Z., Cervinkova, Z., Kucera, O., Gladkova, C., Stopka, P., Krizova, J., Papackova, Z., Oliyarnyk, O., & Kazdova, L. (2015). Metformin prevents ischemia reperfusion-induced oxidative stress in the fatty liver by attenuation of reactive oxygen species formation. American Journal Physiology, Gastroentistinal Liver Physiology, 309(2), 100-111.
48. Leone, A., Lecci, R., Durante, M., Meli, F., & Piraino, S. (2015). The bright side of gelatinous blooms: nutraceutical value and antioxidant properties of three Mediterranean jellyfish (Scyphozoa). Marine Drugs, 13(8),4654-4681.
49. Aluko, R.E. (2015). Amino acids, peptides, and proteins as antioxidants for food preservation. In: Handbook of antioxidants for food preservation. Elsevier. Amsterdam, 105-140.
50. Zhuang, Y., Sun, L., Zhao, X., Wang, J., Hou, H., & Li, B. (2009). Antioxidant and melanogenesis-inhibitory activities of collagen peptide from jellyfish (*Rhopilema*

esculentum). Journal of Science Food Agriculure, 89,17221727.

51. Ding, J. F., Li, Y. Y., Xu, J. J. *et al*. (2011). Study on effect of jellyfish collagen hydrolysate on anti-fatigue and anti-oxidation. Food Hydrocolloids, 25(5), 1350-1353.
52. Biswas, B., Das, B., Jana, K., & Ghosh, D. (2017). Evaluation of antidiabetic and antioxidative efficacy of ethyl acetate fraction of methanolic extract of streptozotocin induced diabetic albino rat. International Journal of Phytomedicine, 9, 305-314.
53. Dludla, P.V., Mabhida, S.E., Ziqubu, K., Nkambule, B.B., Mazibuko-Mbeje, S.E., Hanser, S., Basson, A.K., Pheiffer, C., & Kengne, A.P. (2023). Pancreatic β-cell dysfunction in type 2 diabetes: Implications of inflammation and oxidative stress. World Journal of Diabetes, 14(3),130-146. doi: 10.4239/wjd. v14.i3.130. PMID: 37035220; PMCID: PMC10075035.
54. Han, X., Tao, Y.L., Deng, Y.P., Yu, J.W., Cai, J., Ren, G.F., Sun, Y.N., & Jiang, G.J. (2017). Metformin ameliorates insulitis in STZ-induced diabetic mice. Peer Journal, 13,5, e3155.
55. Lv, Z., Zhang, C., Song, W., Chen, Q., & Wang, Y. (2022). Jellyfish Collagen Hydrolysate Alleviates Inflammation and Oxidative Stress and Improves Gut Microbe Composition in High-Fat Diet-Fed Mice. Mediators Inflammation Journal, 5628702.