Anti-diabetic activity of *Holothuria thomasi* saponin

Amira R. El Barky\textsuperscript{a,\textast}, Samy A. Hussein\textsuperscript{b}, Abeer A. Alm-Eldeen\textsuperscript{c}, Yehia A. Hafer\textsuperscript{d}, Tarek M. Mohamed\textsuperscript{a}

\textsuperscript{a} Biochemistry Unit, Chemistry Department, Faculty of Science, Tanta University, Egypt
\textsuperscript{b} Biochemistry Department, Faculty of Veterinary medicine, Benha University, Egypt
\textsuperscript{c} Zoology Department, Faculty of Science, Tanta University, Egypt
\textsuperscript{d} Chemistry Department, Faculty of Science, Tanta University, Egypt

**Article history:**
Received 5 July 2016
Received in revised form 21 September 2016
Accepted 1 October 2016

**Keywords:**
Streptozotocin
Diabetes
Saponins
Sea cucumber
Oxidative stress

**Abstract**

**Background:** Diabetes mellitus represents a global health problem. It characterized by hyperglycemia that induces oxidative stress leading to a generation of free radicals. A wide variety of natural products in plants and other marine animals represent antioxidant activity and other health benefits like those of sea cucumber. Therefore, this study aimed to investigate the antidiabetic activity of glycosidic compound – saponin – derived from the Egyptian sea cucumber, *Holothuria thomasi*.

**Materials and methods:** Saponin has been extracted from the Egyptian sea cucumber and confirmed by hemolysis, Salkowski tests, FT/IR, UV and GC–MS analysis. Eighty white female albino rats were divided into four equal groups. The first two groups of rats; control normal and control normal saponin-treated groups. The last two groups which were made diabetic by intraperitoneal injection of streptozotocin had one diabetic control and the other diabetic group that got 300 mg/kg B.wt. of saponin extract after Thirty-five days after diabetes induction and lasted for six weeks.

**Results:** The functional group of saponin extract which established with FT/IR spectroscopy demonstrated the presence of saponin in the extracted materials as shown in the peak of the functional group in relevance to the standard one. The UV spectra revealed that $\lambda_{max}$ of saponin extract was 282 nm which in accordance to the standard saponin. Also, GC–MS analysis indicated that the aglycone part of saponin was methyl esters of octadecanoic acid. Saponin extract significantly decreased serum glucose, $\alpha$-amylase activity, adiponectin, IL-6, TNF-$\alpha$ concentrations and liver L-MDA. However, serum insulin and liver glycogen levels were significantly increased as compared with the diabetic non-treated groups. The histopathological results supported that saponin extract markedly reduced the degenerative change in $\beta$-cells.

**Conclusions:** This study, therefore, depicts that the Egyptian *Holothuria thomasi*, sea cucumber saponin as a hypoglycemic agent with the potential to normalize aberrant biochemical parameters and preserved the normal histological architecture of the islets cells of pancreatic tissues.

\(\textcopyright\) 2016 Elsevier Masson SAS. All rights reserved.

1. Introduction

Diabetes mellitus is the most common of the endocrine disorders and it is characterized by chronic hyperglycemia due to relative or absolute lack of insulin secretion or actions [1]. Hyperglycemia which resulted from an accumulation of free glucose in the blood leads to a generation of free radicals due to autoxidation of glucose and glycosylation of proteins [2]. Oxidative stress plays a major role in the diabetic pathophysiology [1]. Also, oxidative stress is thought to be a major factor contributing to the development and progression of diabetic complications, which is also associated with insulin resistance and impaired insulin secretion resulting in the development of diabetes mellitus [3]. Sea cucumber is marine invertebrates characteristic by cylinder-shaped, it lives in different sea floor habitats [4]. Many various bioactive substances such as saponins, polysaccharides, cerebrosides, gangliosides, and phospholipids which characteristic by their rich in nutritional value, potential health benefits and used in chronic inflammatory diseases treatment were found in sea cucumber extract [5]. Various species of sea cucumbers has biological and pharmacological activities including anticancer, anti-hypertension, anti-inflammatory and antioxidant [5].
Moreover, saponins are secondary metabolites synthesized by many different plant species, have high molecular weight glycosides, consisting of a sugar moiety linked to a triterpene or steroid aglycone. Marine organisms such as starfish, sponges and sea cucumbers are now considered a rich source of saponin [6]. Many areas of the world use sea cucumbers in traditional foods and folk medicine [7]. Boiled skin extracts are consumed as a tonic and traditional treatment in Chinese and Malaysian for their effectiveness against Asthma, hypertension, asthma, rheumatism, cuts and burns, impotence and constipation [8,9]. Therefore, there are a lot of research about saponin have been published, most of them focused saponin extracted from plants, only a few of them studied saponin extracted from the marine animal. So, the present study was undertaken to elucidate the anti-diabetic effect of saponin which was extracted from the Holothuria thomasi, sea cucumber on streptozotocin-induced-diabetic rats.

2. Materials and methods

2.1. Preparation of saponins from sea cucumber

Saponin was extracted from the sea cucumber, Holothuria thomasi (purchased from Hurghada, Red sea, Egypt and identified by Zoology department, Faculty of science, Tanta University, Egypt), according to the method described by Hu [10] with some modification. Air-dried body walls (750 g) of sea cucumber were ground into powder and extracted with eight liters of refluxing ethanol. The combined extracts were evaporated with a rotary evaporator and further partitioned between water and chloroform. The water layer was extracted with n-butanol and the organic layer was evaporated with the rotary evaporator to yield n-butanol extracts. The n-butanol extracts were concentrated in drying oven 60°C. The dried butanol extract was 85 g having 93% saponin.

2.2. Identification of extracted saponin

2.2.1. Qualitative erythrocyte hemolysis assay

The presence of saponin in sea cucumber can be detected qualitatively by observed the effect of saponin in the blood. Fresh blood was collected from a healthy child in tubes containing ethylene diamine tetraacetic acid as an anticoagulant. Saponin extract was added and gently mixed with the content. Haemolysis observed by neck eye and under the microscope [11].

2.2.2. Salkowski tests

The two types of saponin can be differentiated by Salkowski Tests [12]. 0.5 g of the dry saponin extracts was added to two ml of chloroform. After shaking very well, sulphuric acid was added slowly to the sides of the test tube to the chloroform layer. Formation of red-brown color indicated the presence of triterpenoid saponin.

2.2.3. Fourier transform infrared (FT/IR)

Dried sea cucumber saponin extract and commercial standard saponin (Fisher Scientific, UK. Saponin, extra pure, SLR by Laboratory Reagents, Legacy Product Code: S/0380/48, CAS Number:8047-15-2) were powdered and analyzed as potassium bromide (KBr) pellets using FT/IR (Model-JASCO FT/IR 4100 LE, made in Japan; Range: 4000–400 cm\(^{-1}\)) [13].

2.2.4. Determination of the maximum wavelength (\(\lambda_{\text{max}}\)) by using ultraviolet spectroscopy (UV)

Maximum wavelength of a compound is unique and so it can be used for qualitative determination of a compound. Saponin extract and commercial standard saponin 100 mg were weighed, dissolved in four ml of distilled water and measured by Shimadzu UV–vis double beam spectrophotometer-160A UV–vis Recording Spectrophotometer Manual. in the UV region (200 nm–650 nm). Then, the graph was plotted between absorbance and wavelength.

2.2.5. Gas chromatography–Mass spectrometry (GC–MS) analysis

Saponin was hydrolyzed with 2 Normal HCL (six h at 100°C) under reflux, the residue was evaporated and the mixture was dissolved in water and extracted with chloroform. The chloroform layer was evaporated to afford the aglycone [14]. Gas Chromatography–Mass Spectrometry analysis was carried out for the hydrolysis of sea cucumber saponin [15], using Perkin Elmer, Clarus 600 gas chromatograph combined to Perkin Elmer, Clarus 600 T Mass Spectrometer.

2.3. Experimental animals

Eighty white female albino Wistar rats, 3–4 months old and average body weight 180–220g were used in the experimental investigation of this study. Rats were purchased from Institute of Ophthalmology, Nasser Eye Institute, Cairo, Egypt. Animals were housed in a room under controlled temperature and a 12 h light-dark cycle with free access to water and chow (EL Haramen for poultry feeding, Quesna, EL Monfia). Rats were handled according to the suggested National ethical guidelines for the care of laboratory animals as the Animal Ethics Committee (IA EC) of Faculty of Science, Tanta University. The animals were left for two weeks for acclimatization before the beginning of the experiment.

2.4. Induction of diabetes

Diabetes in rats was induced by a single intraperitoneal (I.P.) injection of a freshly prepared solution of 40 mg/kg body weight of streptozotocin dissolved in 0.1 M citrate buffer, pH4.5 [16]. A week later, rats with blood glucose concentration higher than 250 mg/dl were considered diabetic [17].

2.5. Experimental design

Saponin extract was dissolved in distilled water for animal treatment [18]. The animals rats were divided into four groups, each of which contained 20 rats.

Group I (G. I): The control normal group. Normal healthy rats received no drugs, served as the normal control for all experimental groups.

Group II (G. II): The control normal treated group received 300 mg/kg B.wt. of saponin extract by gavage syringe at 7:30 a.m. daily.

Group III (G. III): The diabetic control group. (rats were made diabetic by a single i.p., dose of STZ, 40 mg kg-1 body weight).

Group IV (G, IV): The diabetic group treated with saponin extract (300 mg/kg B.wt.) [19], by gavage syringe at 7:30 a.m. daily.

N.B: After STZ induction, rats with stable hyperglycemia were selected for further study. Thirty-five days after diabetes induction therapeutic treatment with saponins extract (300 mg/kg body weight/day) was administered orally and continued for six weeks. All rats groups mention above was subdivided into two groups. Seven rats of each group were sacrificed at the end of four and six weeks of the experiment after the cancellation of dead rats during the experiment.

2.6. Sampling

By the end of the experiment, after four and six weeks from the onset of treatment of saponin extract, random blood samples were collected from all animal groups after overnight fasting by ocular
vein puncture at the end of each experimental period and serum was separated by centrifugation at 3000 rpm for 15 min. The clean and clear serum has proceeded directly for glucose and lipid profile determination and was kept in a deep freeze at −20°C until used for subsequent biochemical analysis. Also, liver and pancreatic tissues specimens were collected.

2.7. Estimation of biochemical parameters

2.7.1. Biomarkers

Serum glucose was estimated enzymatically [20], using kit obtained from Spectrum diagnostics, Egypt. The serum insulin was measured by ultrasensitive rat insulin ELISA kit (Mercodia AB, Uppsala, Sweden). The activities of serum α-amylase was estimated [21], by using commercially available kits (obtained from Spectrum diagnostics, Egypt) and the level of adiponectin in the serum was determined [22], using an Enzyme-Linked Immunosorbent Assay (ELISA) kit according to the manufacturer’s instructions (BioVendor Laboratory Medicine, Inc. Brno, Czech Republic), using a multi-plate ELISA reader (Biorad-680, Biorad Ltd., Japan).

2.8. Determination of serum lipid profile

Total cholesterol and Triacylglycerol were determined enzymatically [23,24] respectively, using kit obtained from Spectrum diagnostics, Egypt.

2.9. Determination of serum kidney functions test

Urea levels in serum was determined enzymatically [25] and serum Creatinine concentration was determined by kinetic method [26] using kit obtained from Spectrum diagnostics, Egypt.

2.10. Determination of serum proinflammatory marker

Serum IL-6 and TNF-α were performed according to [27,28] respectively using ELISA kit (ALP CO Diagnostics, Salem, NH, USA).

2.10.1. “ELISA” part technique

Briefly, 0.1 ml of standards and samples of INS, adiponectin, IL-6 and TNF-α were pipette and incubated into 96-well plates. 0.1 ml of a biotinylated detection polyclonal antibody specific for INS, adiponectin, IL-6 and TNF-α were added, incubated and followed by washing with 0.01 M PBS. 0.1 ml of Avidin-Biotin peroxidase complex was added to each well, incubated and unbound conjugates were washed with 0.01 M PBS. 90 μl TMB substrate solution was added to each well and incubated in dark for 25 min and color intensity develops in proportion to the amount of INS, adiponectin, IL-6 and TNF-α respectively bound in the initial step. The color development is stopped by adding 0.1 ml of TMB stop solution. The intensity of the color is measured at 450 nm.

2.11. Tissue samples (liver and pancreas)

The liver tissues collected at the end of fourth and sixth weeks of the experiment and liver homogenate (10%) was prepared in ice-cold 0.067 phosphate buffer using a chilled glass-Teflon porter-Elvehjem tissue grinder tube. Then the homogenate was centrifuged at 4000 rpm for 10 min at 4°C. The resulting supernatant was used to determine the liver L-MDA and catalase activity. In addition; three rats of each group were sacrificed and their pancreas tissues were immediately fixed in 10% neutral buffered formalin for 24 h.

2.12. Determination of hepatic L-MDA

Liver L-MDA were determined by adding 0.5 ml of trichloroacetic acid (20%) and one ml thiobarbituric acid (0.67%) to 0.5 ml of the liver homogenate (10%). The mixture was heated to 100°C for 15 min, after cooling four ml n-butanol was added, then the mixture was centrifuged at 3000 rpm for 15 min. The absorbance of the resultant color product was measured at 532 nm [29].

2.13. Determination of hepatic catalase activity

Liver catalase activity was estimated [30]. Briefly, 10 μl of liver homogenate was added to the working buffer (160 μl from H2O2 30% was added to each 100 ml Phosphate buffer pH 7 (0.067 M), mixed well by inversion and the change in H2O2 absorbance within 60 sec. was measured at 250 nm.

2.14. Determination of hepatic glycogen

One gram of liver tissue was homogenized in 10 ml of 30% (w/v) potassium hydroxide and was set to boil at 100°C for 30 min. The solution was treated with ethanol to precipitate glycogen which was pelleted, washed and redissolved in distilled water. Absorbance was measured at 492 nm using a multi-plate ELISA reader [31].

2.15. Histological examination

The pancreas tissues were immediately fixed in 10% neutral buffered formalin for 24 h.

The samples were then dehydrated, cleared and embedded in paraffin and sectioned at 5 μm. Some slices were dewaxed, rehydrated and processed for hematoxylin and eosin staining and others were processed for immunostaining. For immunohistochemistry, the sections were further washed in distilled water for 5 min then rinsed in 0.01 M phosphate buffered saline (PBS), pH 7.3 for 10 min. The sections then were incubated with insulin guinea pig-human antiserum (N. Yanaihara, Shizuka, Japan, 1:3000) for two hours at room temperature then rinsed in PBS. The sections were further incubated with guinea pig immunoglobulin conjugated with peroxidase (Dako A/S, Denmark, 1:200) for one hour at room temperature then rinsed in PBS. The reaction was developed as a brown color using 3-3’ diaminobenzidine tetrahydrochloride (DAB, Wako pure chemical industries, Ltd) in 40 ml PBS, pH 7.2 containing 10 ml of hydrogen peroxide for 10 min in a dark room. The section was rinsed in distilled water and counterstained with Mayer’s HX, hydrated in ascending grades of alcohol, cleared and mounted [32].

2.16. Statistical analysis

The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple tests All analyses were performed using the statistical package for social science [33]. Values of P ≤ 0.05 were considered significant.

3. Results

3.1. Confirmatory tests of extracted saponin

Healthy child blood sample hemolysis as a confirmation for the presence of saponin in sea cucumber extracted material. Whereas, the presence of red-brown layer at the lower interface after addition of chloroform and concentrated H2SO4 investigated that the extracted saponin was of triterpenoid type.
3.2. Fourier transform infrared spectroscopy (FT/IR)

The functional groups which detected by FT/IR spectrum (4000 and 400 cm⁻¹) of isolated saponin showed that the peaks of functional groups are in compatible to those of the reference standard one (Fig. 1).

3.3. Ultraviolet spectroscopy (UV)

Ultraviolet spectra of the extracted saponin indicated that saponin extract has λ_max at 282 nm, likewise to those of the standard saponin which revealed the presence of λ_max at 283,305 nm Fig. 2.

3.4. The GC–MS analysis

The GC–MS analysis of the extracted saponin showed that the aglycone moiety composed of two prominent peaks as the main component of saponin extract at retention time 24.796 and 24.851 with biggest abundance of 58.24% and 22.68%, respectively which also confirmed that it was a triterpenoid type Figs. 3 and 4).

3.5. Effect of saponin extract on serum biomarkers glucose, insulin, α-amylase and adiponectin

In order to ensure the hyperglycemia state, the serum glucose and insulin were assessed. The obtained data in Figs. 5 and 6 showed an incredible increase in the serum glucose. Meanwhile, there was a significant decrease in serum insulin in STZ-induced diabetic female rats(G. III) compared to the control normal group (G. I) after four and six weeks of the experiment. On the other hand, Administration of Saponin to STZ-induced diabetes female rats significantly reduced elevated serum glucose level and increase serum insulin levels after four weeks of the experiment but it didn’t reach the normal value as compared to the control normal group (G. I). Also, after six weeks of the experiment, there was a significant decrease in serum glucose and a significant increase of serum insulin in diabetic saponin treated group (G. IV) as compared with the control diabetic non-treated group (G. III) While, there was a detection of the normal value as compared to the control normal group (G. I). Also, the results pertaining to S. α-amylase activity (Fig. 7) showed a nonsignificant change after four weeks of the experiment of STZ-diabetic non-treated groups (G. III) as compared to control normal group (G. I). While, after experimental six weeks a significant increase was observed as compared to the normal control group (G.I). On the other hand, diabetic saponin-treated rats non-significantly reduced the elevation of serum α-amylase activity after experimental four weeks. Meanwhile, after six weeks there was a significant decrease as compared to those of the STZ-non-treated group (G. III) and a nonsignificant change was observed as compared to the control normal group (G. I). In addition, S. adiponectin concentration in STZ-diabetic non-treated rats (G. III) showed a significant increase after four and six weeks of the experiment as compared to the control normal group (G. I). Meanwhile, diabetic saponin-treated rats (G. IV) showed a significant decrease of serum adiponectin concentration after four and six weeks of the experiment in comparison to the diabetic non-treated group (G. III) and it returned back to the normal value in comparison with the control normal group (G. I) Fig. 8.

3.6. Effect of saponin extract on serum lipid profile parameters

Serum total cholesterol and Triacylglycerols levels which detected on different treated groups showed significantly increase (P < 0.05) in serum total cholesterol and S. triacylglycerols concentration after experimental four weeks in STZ-induced diabetic rats (G. III). Meanwhile, a significant decrease was observed after six weeks as compared to those of the control normal group (G. I).Conversely, diabetic saponin-treated group (G. IV) showed a significant reduction of both sera total cholesterol and TG concentration after four weeks of the experiment as compared to STZ-diabetic non-treated group (G. III) and reached the normal value as compared to the control normal group (G. I). On the other hand, there was a significant increase after six weeks of the experiment as compared to the STZ-diabetic non-treated group (G. III) and showed a nonsignificant change in comparison to control normal group (G. I) Figs. 9 and 10 respectively.

3.7. Effect of saponin extract on kidney function tests

Regarding the results of urea and creatinine levels. Serum urea levels (Fig. 11) showed a significant increase in STZ-induced diabetic rats (G. III) after four and six weeks of the experiment as compared to those of the control normal group (G.I). On the other hand, diabetic saponin-treated rats (G. IV) resulted in a significant decrease of S. urea levels after four weeks of the experiment as

---

**Fig. 1.** FT/IR spectrum of the saponin extract and reference standard saponin.
compared to those of the STZ-diabetic non-treated group (G. III) but it didn't reach the normal value as compared to the control normal group (G. I). Meanwhile, after six weeks of the experiment there was a significant increase of S. urea levels in (G. IV) in comparison to both STZ-diabetic non-treated group (G. III) and the control normal group (G. I). In addition, S. creatinine levels which detected in differently treated group showed a nonsignificant change in all studied groups after four weeks of the experiment as compared to the control normal group (G. I). However, a significant increase of S. creatinine levels in the STZ-induced diabetic rats (G. III) was observed after six weeks of the experiment when compared with the control normal group (G. I). On the other hand, diabetic saponin-treated rats (G. IV) showed a significant reduction of S. creatinine levels as compared to the STZ-diabetic non-treated group (G. III) and showed a nonsignificant change when compared with the control normal group (G. I) Fig. 12.

3.8. Effect of saponin extract on proinflammatory marker

Depicts the results of the serum pro-inflammatory cytokines IL-6 and TNF-α, there was a significant increase after four and six weeks of the experiment in the STZ-diabetic rat groups (G. III) when compared with the control normal group (G. I). Meanwhile, diabetic saponin treated rats (G. IV) showed a significant reduction in the values of serum IL-6 after four and six weeks of the experiment when compared with the STZ-diabetic non-treated group (G. III). but it didn't reach the normal value as compared to the control normal group (G. I). Moreover, diabetic saponin-treated rats (G. IV) showed a significantly reduction of S. TNF-α concentrations after four and six weeks as compared to the diabetic non-treated group (G. III) and it returned back to the normal value after four weeks of the experiment in comparison to control normal group (G. I). Meanwhile, a significant increase was observed after six weeks compared to the control normal group (G. I) Figs. 13 and 14 respectively.

3.9. Effect of saponin extract on lipid peroxidation and catalase activity

The hepatic levels of L-MDA, as a biomarker of lipid peroxidation, in STZ-induced diabetic rats (G. III) were statistically higher (p ≤ 0.05) than those of the control normal group (G. I) after four and six weeks of the experiment Fig. 15. Orally administration of Saponin to the STZ- induced diabetic rats (G. IV) non-significantly reduced elevated Liver L-MDA after four and six weeks of the experiment compared to the STZ-diabetic non-treated group.
Fig. 5. Effect of saponin extract administration on the levels of serum glucose (mg/dl) of STZ-diabetic rats. *Significantly different from normal and normal + saponin. ** Significantly different from normal, normal + saponin and STZ-diabetic group (P ≤ 0.05).

Fig. 6. Effect of saponin extract administration on the levels of serum insulin (nIU/ml) of STZ-diabetic rats. *Significantly different from normal and normal + saponin. ** Significantly different from normal, normal + saponin and STZ-diabetic group (P ≤ 0.05).
Fig. 7. Effect of saponin extract administration on the activities of Serum α-amylase (U/L) of STZ-diabetic rats. *Significantly different from normal and normal + saponin (P ≤ 0.05).

Fig. 8. Effect of saponin extract administration on the levels of serum adiponectin level (pg/ml) on STZ-diabetic rats. *Significantly different from normal and normal + saponin (P ≤ 0.05).
Fig. 9. Effect of saponin extract administration on the levels of serum cholesterol level (mg/dl) on STZ-diabetic rats. *Significantly different from normal and normal + saponin (P ≤ 0.05).

Fig. 10. Effect of saponin extract administration on the levels of serum Triacylglycerol (mg/dl) on STZ-diabetic rats. *Significantly different from normal and normal + saponin (P ≤ 0.05).
Fig. 11. Effect of saponin extract administration on the levels of serum Urea (mg/dl) on STZ-diabetic rats. *Significantly different from normal and normal + saponin. **Significantly different from normal, normal + saponin and STZ-diabetic group (P ≤ 0.05).

Fig. 12. Effect of saponin extract administration on the levels of serum creatinine (mg/dl) on STZ-diabetic rats. *Significantly different from normal and normal + saponin (P ≤ 0.05).
Fig. 13. Effect of saponin extract administration on the levels of serum IL-6 (pg/ml) on STZ-diabetic rats. *Significantly different from normal and normal + saponin. **Significantly different from normal, normal + saponin and STZ-diabetic group (P ≤ 0.05).

Fig. 14. Effect of saponin extract administration on the levels of serum TNF-α (pg/ml) on STZ-diabetic rats. *Significantly different from normal and normal + saponin. **Significantly different from normal, normal + saponin and STZ-diabetic group (P ≤ 0.05).
Fig. 15. Effect of saponin extract administration on the levels of Liver L-MDA (mmol/gm tissue) on STZ-diabetic rats. *Significantly different from normal and normal + saponin (P ≤ 0.05).

Fig. 16. Effect of saponin extract administration on the activities of Liver Catalase (mmol/min/mg protein) on STZ-diabetic rats. *Significantly different from normal and normal + saponin (P ≤ 0.05).
Meanwhile, intact a and compared experiment after six weeks when compared with the control normal group (G. I). Saponin extract treatment nonsignificantly increases liver catalase activity in the STZ-diabetic group (G. IV) after six weeks of the experiment as compared to the STZ-diabetic non-treated rats (G. III) and showed a nonsignificant decrease as compared to the control normal group (G. I) Fig. 16.

3.10. Effect of saponin extract on hepatic glycogen

Liver glycogen levels that have been detected on different treated groups showed a nonsignificant change in the STZ-induced diabetes female rats group (G. III) after four and six weeks of the experiment as compared to the control normal group (G. I). Meanwhile, diabetic saponin-treated rats (G. IV) resulted in a significant increase of liver glycogen after four and six weeks of the experiment as compared to the STZ-diabetic rat non-treated group (G. III) and compared to the control normal group (G. I) Fig. 17.

Furthermore, saponin extract administered normal rats (G. II) for four and six weeks showed no significant changes in all serum and liver tissue parameters when compared with the control normal groups (G. I).

3.11. Histopathological findings

Observation of the pancreatic sections of the control rats showed intact Langerhans’ islets with well-defined edges and plump islets cells. The sections which were stained with insulin antisera revealed that β-cells were easily distinguishable in most islets especially in its peripheral parts (Fig. 18 G. I.G. II and Fig. 19a,b.

Examination of the pancreatic sections of the diabetic rats showed an extensive destruction of the Langerhans’ islets. The islets had ill-defined boundaries and some necrotic areas were often noticed. Severe inflammatory cells infiltrations were observed. Some endocrine cells had vacuolization in their cytoplasm and others had no cytoplasmic secretion. Diabetic pancreas which was stained with insulin antisera displayed a profound reduction in the β-cells (Fig. 18 G. III.4, GIII. 6 and Fig. 19c). The sections from the diabetic rats which were treated with saponin restored somewhat the normal appearance of the Langerhans’ islets. In addition, the sections which were stained with insulin antisera showed normal detection with β-cells although their numbers were still less than what was observed in the control islets (Fig. 18 G. IV4.G. IV6 and Fig. 19d).

4. Discussion

Saponins are the main bioactive compounds in sea cucumber that exhibit a wide range of biological activities and have many therapeutic effects [34]. The reaction of the saponin with cholesterol which occur in the erythrocyte wall cause permeability changes which it may be responsible for the hemolytic activity [35]. This confirmed the current results which indicated hemolysis the blood of a healthy individual. The identification of hydroxyl, alkyl, ether and ester groups of isolated triterpenoid saponin hederacoside C in the FTIR spectrum indicated the presence of saponin [36]. These confirmed the obtained results which showed that, a long sharp peak at 3400.10 cm⁻¹ indicates the presence of hydroxyl groups (–OH), and the peak at 2928.85 cm⁻¹ represents alkyl groups (C–H), C=C at 1408, C=O stretching of carbonyl group at 1641 sharp beak and Oligosaccharide linkage absorption to sapogenins, that is C–O–C were apparent between 1054 and 1261.08 cm⁻¹. Most of the saponins exhibit major absorption peaks in the range of 250–350 nm. The present results indicated that UV

![Graph](image)

Fig. 17. Effect of saponin extract administration on the levels of Liver glycogen (µg/mg tissue) on STZ-diabetic rats. *Significantly different from normal, normal + saponin and STZ-diabetic non-treated group (P ≤ 0.05).
spectra of sea cucumber saponin have $\lambda_{max}$ at 282 nm, this results were in agreement with Gonzalez-Valdez et al. [37] who stated that UV spectra of saponin extracts of Agave durangensis foliar tissue showed four types of saponin structures which were detected and all presenting a major absorption band at 275 nm. The results which concerning to GC–MS analysis of the extracted saponin showed two prominent peaks as the main component of saponin extract, was methyl ester of octadecanoic acid which had the biggest abundance of 48.4% and 18.85%, respectively. This indicated that the saponin extracted from Holothuria thomasi, sea cucumber from tritterpenoid type. Some bioactive compounds were recognized in methanolic extract of sea cucumber Stichopus variegates which included 2-carbamoyl-3-methylquinoxaline, butanal-3-methyl,octadecanoic acid methyl ester, 2-methyl-7-phenylindole and heptanoic acid methyl ester [38].

Diabetes is a serious complex chronic condition that is a major source of ill health worldwide. Streptozotocin is used to prompt experimental diabetes in rodents and inducing hyperglycemia in the studies of diabetes [39,40]. STZ is a toxin with the ability to induce selective destruction of pancreatic beta cells resulting in insulin deficiency and hyperglycemia [41]. A number of diabetic animal models have been developed and improved over the years [42]. Saponin extract treatment to diabetic rats showed a significant decrease in serum glucose and significantly increase serum insulin levels as compared with the diabetic non-treated group (G.III). Saponins have been reported to decrease blood glucose level [43] which occur in different mechanisms such as regeneration of insulin action via increased plasma insulin level and release insulin from the pancreas [14] and blocks the formation of glucose in the bloodstream [44].

Alpha-amylase is one of the main enzymes produced in exocrine pancreatic cells, may be recognized as an adequate indicator of organ's activity both in physiological and pathological states [45]. The obtained results demonstrated that a significant increase in serum $\alpha$-Amylase activity was observed in serum STZ-diabetic rats (G. III) after six weeks of the experiment when compared to the control normal non-treated group (G. I). The increment in enzyme activity may reflect the release of the enzyme from cellular compartments caused by increased damaging processes [44]. The decrease in serum $\alpha$-amylase activity which was observed in the saponin-treated group (G. IV) after six weeks of the experiments may be Attributed to that saponins have an inhibitory activity against the carbohydrate hydrolyzing enzymes intestinal $\alpha$-glucosidase and pancreatic $\alpha$-amylase [46].

There was a significant increase in serum adiponectin concentration in streptozotocin-induced diabetic rats (G. III) after
four and six weeks of the experiments when compared with the control normal group (G I). The increase in adiponectin levels in type 1 diabetes appears to be strongly associated with long diabetes duration, irrespective of the metabolic control. Among other factors, a putative role for residual beta-cell function in the regulation of circulating adiponectin levels can be considered [47].

Saponin treatment to STZ-diabetic rats resulted in a significant decrease of serum adiponectin concentration after four and six weeks of the experiment when compared with the diabetic non-treated group (G. III). The main mechanism by which adiponectin enhances insulin sensitivity appears to be due to improved lipid and glucose metabolism [48].

Insulin plays an important regulatory role in lipid and glucose metabolism [49]. The absolute or relative lack of insulin as seen in diabetes leads to abnormal metabolism of lipid and carbohydrate. The obtained results showed a significant increase in serum total cholesterol and triacylglycerols concentrations after four weeks in the diabetic non-treated groups (G. III). The level of serum lipids is usually increased in diabetes due to the increase of blood glucose and such an elevation represents a risk factor for coronary heart disease [50]. Serum total cholesterol and triacylglycerols concentrations were markedly decreased in saponin extract treated diabetic rats. Saponin may probably change the rate of fatty acids oxidation in the liver and reduced the rate of triglycerides biosynthesis in rats [51]. On the other hand, a significant decrease in both serum total cholesterol and triacylglycerols of the STZ-diabetic non-treated group were observed after six weeks when compared with the normal control group (G.I). The decrease of lipid profile may be due to rats need more energy rather than glucose so, it broke down lipids to obtain energy which in agreement with EL Barky [52]. Meanwhile, saponin treatment significantly increases both serum cholesterol and triacylglycerols which it may be due to decrease glucose levels and increase levels of insulin which help the female rats to store lipids again in their body.

The obtained data showed a significant increase in both sera urea and creatinine levels. The urea and creatinine levels have been considered a significant biomarker of diabetic nephropathy which increased in the STZ-induced diabetic group [53]. The elevation levels of serum creatinine and urea were consistent with the impaired kidney function [54]. An increased urea concentration in diabetic rats is associated with greater protein catabolism. Serum urea was markedly decreased in the saponin-treated group (G.IV) after four weeks and serum creatinine was decreased after six weeks of the experiment. The presence of natural antioxidants such as saponins has been reported to exhibit a major role in reducing oxidative stress associated with diabetes, which in turn helps in the regulation of normal kidney functions [55]. On the other hand, there was a significant increase in serum urea levels in saponin treatment group (G. IV) after six weeks when compared with the diabetic non-treated group (G. III) and the control normal group (G. I). These results were in agreement with [56], who stated that serum urea concentration was significantly higher in both the diabetic STZ and STZ-treated with saponins groups in comparison with the controls.

As markedly, from the results obtained in Figs. 13 and 14, there was a significant increase in serum pro-inflammatory cytokines IL-6 and TNF-α concentrations in the diabetic rat group (G. III). TNF-α, Interleukin-1 and IL-6 produced by infiltrating macrophages, lymphocytes, and monocytes, damaged the pancreatic β-cells and produced type-1 DM by enhancing the formation of oxygen free radicals, lipid peroxides and aldehydes [57,58]. Meanwhile, oral saponin extract administration in STZ-diabetic rats (G. IV) resulted in a significant reduction in the values of serum IL-6 and TNF-α concentrations. Ginsenosides saponins can inhibit the lipopolysaccharide-induced production of TNF-α by blocking transcription factor NF- KB (nuclear factor kappa-light-chain-enhancer of activated B cells) which regulates the transcription of many genes associated with inflammation [59].

Reactive oxygen species directly oxidize and damage DNA, protein and lipids and plays a key direct role in the pathogenesis of late diabetic complications [60]. The present study showed that a significant increase of liver L-MDA concentration in the STZ-diabetic rat group (G. III) compared to the control normal group (G. I). Meanwhile, oral administration of saponin extract to STZ-diabetic rats significantly reduced liver L-MDA as compared to the diabetic non-treated group. The obtained results are nearly similar to Elekofehinti [51] who reported that treatment with Saponin extract from Solanumanguivi anguivi fruit for 21 days significantly lowers MDA concentration in the serum, liver, and pancreas. The reduction of MDA levels could be attributed to the antioxidant activity of saponins [51]. Also, saponin has been
reported to be good chelator of metals [51], hence making these metals unavailable for glucose autoxidation. Also, the presence of many OH groups in the structure of saponin is responsible for raising antioxidant activity and this property is responsible for the hindering of ROS formation in diabetes. In the current work, a nonsignificant increase in liver catalase activity was observed in the diabetic rats treated with saponin extract compared to the diabetic rat non-treated group. The obtained results are nearly similar to [51] who demonstrated that the levels of the antioxidant enzymes SOD and CAT were significantly saved after saponin treatment, proposed that saponins have effective anti-oxidative properties and could scavenge excess free radicals. Consequently, saponin may have a functional protective mechanism in response to ROS which may help to regenerate β-cells and save pancreatic islets against cytotoxic effects of alloxaon [51].

Saponin extract treatment to the STZ-diabetic rats resulted in a significant increase of liver glycogen after four and six weeks of the experiment when compared with STZ-diabetic non-treated group. Saponins have anti-diabetic activity and the anti-diabetic activity may be due to reversing of the atrophy of the pancreatic islets of β-cells, as a result of which there may be increased insulin secretion and increased the hepatic glycogen level [61].

The present biochemical results parallel the histological observations of the endocrine pancreas. The insulin-producing beta cells were degraded and lowered in number in STZ-diabetic group (G.III), resulting in diminished serum insulin concentration. Also, the immunohistochemical findings confirm the results reported by Abunasef [62] who found a decreased number of insulin immunoreactive cells in the pancreatic islets of diabetic rats. The results could be explained by considering the mode of action of STZ, which stimulates an increased release of oxygen species, thereafter causing DNA damage [63]. This damage leads to activation of DNA repair enzyme poly-ADP-ribose polymerase-1 (PARP-1). In consequence, a reduction in intracellular NAD is followed by ATP depletion, which leads to pancreatic β-cell death [63]. The pathological improvement in the pancreas of STZ-induced diabetic rats treated with saponin extract compared to the diabetic non-treated group was confirmed also by both the histological observations and the immunohistochemical findings. These results could be attributed to the hypoglycemic action and antioxidant potential of saponin extract treatment in diabetic rats. As confirmed by the finding of [64] who stated that saponin isolated from Momordica cymbalaria possess potential anti-diabetic activity as it lowers blood glucose level and has shown to improve beta cell density.

5. Conclusion

Saponin extracted from sea cucumber Holothuria thomasi, contain methyl ester of octadecanoic acid as aglycone part and their function groups are the same of the commercial standard saponin. Sea cucumber saponin extract may be effective in controlling glycemic status, improving dyslipidemia, has a potential anti-inflammatory and antioxidant activities as well as diminishing diabetic cardiovascular complications.

Acknowledgement

I wish to express my sincere thanks and deepest to Prof. Dr. Ehab Mostafa Mohamed Ali Professor of Biochemistry, faculty of Science Tanta University, for his continuous help and cooperation.

References

the administration of saponins from Solanum anguivi fruits exhibit hypolipidemic potential in Rattus norvegicus, Der Pharmacia Lett. 4 (2012) 811–814.

A.R. El-Barky, Biochemical Influence of Alpha-lipoic Acid on Lipid Peroxidation and Antioxidant Enzymes in Blood and Tissues of Streptozotocin Induced Diabetes in Rats, Faculty of Veterinary Medicine, Moshith, Benha University, 2012 (Ms. thesis).


