



Saponin ameliorate diabetes in STZ-diabetic rats

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Abstract: Saponins exhibit a wide spectrum of biological activities. Hyperglycemia was induced by STZ. Saponin extract(300 mg/kg B.wt.) was administrated to STZ-diabetic rats. Saponin extract significantly reduced serum blood glucose, cholesterol, triacylglycerol, liver L-MDA and significantly increased serum insulin level, In conclusion, saponin has antidiabetic effect on STZ- diabetic rats.

Key words: Streptozotocin, Diabetes, oxidative stress, Saponin.

Introduction

Diabetes mellitus is a metabolic syndrome, has growing problem worldwide(Khattab *et al.*,2015).The number of diabetes will jump from 382 million in year 2013 to 592 million in year 2035(Sheweita *et al.*, 2016).Hyperglycemia is the characteristic form of diabetes mellitus which resulted from accumulation of free glucose in the blood. It leads to generation of free radicals due to autoxidation of glucose and glycosylation of proteins (Shahidi *et al.*, 2016).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. They generate stable foam and lyse blood cells because of their amphiphilic nature (Moghimpour *et al.*, 2014). Marine organisms such as starfish, sponges and sea cucumbers are now considered a rich source of saponin (Moghimpour *et al.*, 2015).Sea cucumber are marine invertebrates, they are cylinder-shaped that live in a variety of sea floor habitats from warm tropical

waters to cold deep-sea trenches (Dakrory et al., 2015). The extract of the sea cucumber characteristic by High nutritional value, potential health benefits and used in chronic inflammatory diseases treatment. Therefore, the present study was aimed to evaluate the anti-diabetic potential of saponin extracts of sea cucumber in STZ- induced diabetic rats.

Materials and methods

Preparation of saponins:

Saponins were isolated from the sea cucumber, *Holothuria thomasi* (purchased from Hurghada, Red sea, Egypt and identified by Zoology department, Faculty of science, Tanta, Egypt), according to Hu et al., (2010)

Identification of saponin by FT/IR:

Dried saponin extract and standard saponin were powdered and analyzed as potassium bromide pellets using FT/IR.

Experimental animals:

forty white female albino rats (3-4 months old and average body weight 180-220 g) were housed in a room under controlled temperature and a 12h light-dark cycle with free access to water and chow.

Induction of diabetes:

Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared solution of 40 mg /kg of streptozotocin (Tariq et al., 2013). A week later, rats with blood glucose

concentrations higher than 250 mg /dl were considered diabetic and included for further studies (Cheng et al., 2013).

Experimental design:

Rats were divided randomly into 4 groups (10 rats per group), Group (I) served as a normal control which were received no drugs. Group (II) Normal healthy rats which were received extracted 300 mg/kg/day of saponin extract, Group (III) diabetic rats and Group (IV) Diabetic rats which were received 300 mg/kg/day of saponin extract. At the end of the experiment, rats were fasted overnight. blood samples were collected by ocular vein puncture. The animals were then scarified and the liver were removed.

Biochemical investigation

Serum glucose was measured by enzymatic colorimetric method using kit obtained from Spectrum diagnostics, Egypt. Serum insulin was determined by ultra-sensitive rat insulin ELISA kit. HOMA-IR was calculated using the formula described by (Haffner et al., 2002). β -cell function was calculated using the formula described by (Matthews et al., 1985). Total cholesterol and Triacylglycerols in serum was determined using reagent kits purchased from, Spectrum diagnostics. Liver Tissues were dissected out, washed in ice-cold saline, weighed and 10% tissue homogenate was prepared. Liver L-MDA was

estimated according to (Mesbah et al., 2004), total protein thiol(Sedlak and Lindsay, 1968) and liver glycogen was estimated according to the method described by Miikue-Yobe Togenu et al.,(2013).

Statistical analysis:

The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. Values of $P \leq 0.05$ were considered to be significant.

Results

The IR spectrum of isolated saponin gave identical results as compared with reference standard of saponin.

The levels of serum glucose were significantly increased whereas serum insulin levels were significantly decreased in diabetic rats compared to normal control. Diabetic rats treated with saponin showed a significant decrease in serum glucose level and a significant increase in serum insulin levels by the end of the experimental period compared with(G.III). On the other hand, a significant increase in serum HOMA-IR concentration and a significant decrease of serum β cell function level was observed in diabetic rats when compared with normal control group. Saponin treatment(G.IV) resulted in a significant

decrease of serum HOMA-IR level and a non significant increase of serum β cell function level when compared with diabetic non-treated group (table1). The levels of lipid profile in the control and experimental groups of rats were shown in Table2. The obtained data showed a significant increase in both total cholesterol and TG in diabetic rats when compared with control normal group. Meanwhile, the treatment of diabetic rats with saponin extract resulted in a significant reduction of both serum cholesterol and TG when compared with diabetic group. The hepatic levels of MDA were statistically higher than those of normal control group. and diabetic rats treated with saponin showed a non significant change in liver protein thiol when compared with diabetic group. On the other hand, liver glycogen showed a non significant change In diabetic group when compared with normal control group. Meanwhile, Saponin treatment resulted in a significant increase of the level of glycogen when compared with diabetic group(table3). Our results revealed that, a significant increase in both liver and kidney weights was observed in both diabetic rats and diabetic rats treated with saponin (Table 4).

Table1. Effect of saponin extract supplementation on serum glucose, Insulin levels and in normal and STZ-induced diabetic rats.

Animal groups	Glucose (mg/dL)	Insulin nIU/ml	HOMA-IR	B-cell function
Group I	69.12 ±1.85 ^c	55.55±3.61 ^a	0.001±0.0003 ^c	3.84±0.398 ^a
Group II	72.76±2.10 ^c	47.85±2.81 ^a	0.001±0.0002 ^c	1.70±0.475 ^b
Group III	370.80 ±9.68 ^a	21.96±1.62 ^c	0.017±0.0012 ^a	0.025±0.002 ^c
Group IV	169.40± 3.23 ^b	34.01±3.71 ^b	0.009±0.0013 ^c	0.117±0.011 ^c

Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table2. Effect of treatment with Saponin on serum ureas, creatinine, cholesterol and Triacylglycerols levels in normal and STZ-induced diabetic rats.

Animal groups	Cholesterol (mg/dl)	Triacylglycerols(mg/dL)
Group I	43.88±2.55 ^b	57.09±2.54 ^b
Group II	36.09±4.249 ^b	45.85±1.255 ^b
Group III	115.75±1.91 ^a	285.23±18.90 ^a
Group IV	44.22±1.004 ^b	41.40±1.89 ^b

Table 3: Effect of treatment with Saponin on liver L- MDA,TPT levels and catalase activity in normal and STZ-induced diabetic rats.

Animal groups	L – MDA (nmol/gm tissue) ×10 ⁻²	totalprotein thiol(mmol/gm tissue)	Glycogen µg/g tissue
Group I	0.008±0.001 ^b	0.013±0.001 ^a	0.098±0.03 ^c
Group II	0.013±0.001 ^{ab}	0.012±0.001 ^a	0.083±0.01 ^c
Group III	0.017±0.0003 ^a	0.009±0.001 ^a	0.094±0.02 ^c
Group IV	0.012±0.002 ^{ab}	0.011±0.002 ^a	0.225±0.003 ^a

Table 4. Effect of saponin extract supplementation on liver and kidney weight(g) in normal and STZ-induced diabetic rats.

Animal groups	Liver weight	Kidney weight
Group I	5.47±0.12 ^c	1.39±0.03 ^b
Group II	5.98±0.05 ^b	1.29±0.01 ^b
Group III	6.48±0.07 ^b	1.70±0.04 ^a
Group IV	7.18±0.13 ^a	1.68±0.11 ^a

Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Discussion

Saponins are the main bioactive compounds in sea cucumber that exhibit a wide range of biological activities and have many therapeutic effects (Sottorff et al., 2013). Our results showed that, Saponin treatment significantly reduced elevated serum glucose level in diabetic rats. Saponins have been reported to

decrease blood glucose levels in different mechanisms such as stimulating insulin release in pancreas, reducing hepatic

glucose production, increasing glucose consumption of tissues (Lee et al., 2000)

The obtained results revealed that, Saponin treatment resulted in a significant increase of insulin concentration in group (IV). Saponins

stimulates the release of insulin and blocks the formation of glucose in the blood stream(Rajalakshmi et al., 2015).

Saponins are known anti nutritional factors, which lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase its fecal excretion (Rotimi et al., 2011).

The results of table (3), Saponin treatment resulted in a significant increase of the level of glycogen when compared with diabetic group. This is possibly due to either the stimulation of insulin release from β -cell (Lolitar and Rao,1996)or insulinomimetic activities of the extract giving rise to direct peripheral glucose uptake or a combination of the two.

Conclusion

Our data concluded that, saponin may be effective in controlling glycemic status, improving dyslipidemia and scavenging free radicals.

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تحسن الجردان المصابة بداء السكري باستخدام السابونين
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هدفت هذه الدراسة الى استخلاص الصابونين من خيار البحر وتقييم تأثيره على الجردان المصابة بمرض السكر. هذا وقد أستخدم لأجراء هذه الدراسة عدد 40 من اناث الجردان البيضاء. وقد قسمت إلي أربعة مجموعات متساوية. وقد أسفرت نتائج التحليل البيوكيميائي أن مجموعة الجردان المحدث بها مرض البول السكري والتي تم علاجها بمستخلص الصابونين تسبب في خفض كلا من مستوى الجلوكوز والدهون بالدم بالإضافة إلى نسبة الأوكسدة الفوقية للدهون بالكبد في حين ارتفع مستوى الانسولين في الدم. وخلصت الدراسة أن مستخلص الصابونين له تأثير جيد في خفض مستوى سكر الدم وتحسين نسبة الدهون العالية لذلك لديه القدرة من الحد من مضاعفات أمراض القلب التي تنتج من زيادة الدهون. كذلك فان مستخلص الصابونين لديه القدرة للحد من المضاعفات الناتجة من زيادة الأوكسدة الفوقية للدهون بتخفيضها.