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The Effect of Ginseng on Streptozotocin Induced Diabetic Cardiomyopathy in Male Rats

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Abstract

Background: Diabetes is considered one of the most common metabolic disorders world wide. Diabetic cardiomyopathy is considered a great complicationassociated with diabetes that can affect patients causing mortality and morbidity. Ginseng is one of natural products used in complementary medicine.

Aim of Study: The present study aimed to find out the effect of ginseng on diabetic heart and the possible underlying mechanisms.

Material and Methods: Adult male albino rats with body weights of 180 ~ 200g were introduced in the study; they were divided into: Control normal, Diabetic group, Diabetic + ginseng. At the end of the study blood samples were collected for estimation of blood glucose, insulin, fatty acids, cardiac enzymes, beta catenin, IL-6, caspase 3, glutathione peroxidase and reduced glutathione. Cardiac tissue was collected for histological analysis.

Results: Results showed deterioration in all parameters in diabetic group while on administration of ginseng all parameters were improved.

Conclusion: Ginseng improved diabetic cardiomyopathy and prevents further cardiac deterioration.

Key Words: *Ginseng – Cardiomyopathy – IL-6 – Caspase3 – Glutathione.*

Introduction

DIABETES mellitus (DM) is associated with multiple complications due to the abnormal glucose and fat metabolism. Diabetic cardiomyopathy (DCM), a condition found in diabetic patients, it is considered a major contributor to morbidity and mortality in diabetic patients **[1].** It is characterized by structural and functional changes in the myocardium **[2].** The disease manifests as focal myocardial necrosis, caused by metabolic disturbances and microvascular complications, cardiac dysfunction that progresses to heart failureand sudden death **[1].** Different molecular mechanisms could be attributed in the development of diabetic cardiomyopathy as oxidative stress, inflammation, endoplasmic reticulum (ER) stress and apoptotic cell death **[3].** Mitochondrion is the main "factory" in which DM produces excessive mitochondrial superoxide **[4].** Mitochondrial superoxide leads to increased formation of advanced glycosylation end products (AGEs) activation of protein kinase C (PKC) and the polyol pathway **[5].** Moreover, diabetic complications could be attributed to the inflammatory process that is accelerated under a hyperglycemic conditions **[5].** Beta (β) actin is expressed in all four chambers of the heart as well as in cultured adult cardiomyocytes. Overexpression of β-actin promotes cell spreading in many cell types, and in dilated cardiomyopathy elevated levels of β-actin have been reported **[7].** Red ginseng root (Panax ginseng) as been used clinically in Asian countries for the treatment of various diseases including cerebrovascular disease, hypertension, atherosclerosis, liver dysfunction, and postmenopausal disorders **[8]** as Ginseng is well known nowadays by its antioxidant activity **[9].** Moreover ginseng is relatively safe and inexpensive adjuvant used in treatment of diabetes and chronic diabetic complicationsas ginsenoside Re, Rb1, and Rb2, its active ingredients, have demonstrated to have antidiabetic action and improve glucose metabolism **[10].** To our knowledge few studies searched for the role of ginseng on the progression of diabetic cardiomyopathy in rat model of diabetic cardiomyopathy.

Aim of the work:

Thus we aimed in this study to find out the role of red ginseng extract in amelioration of diabetic cardiomyopathy and elucidate the possible underlyingmechanisms.

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Material and Methods

Experimental animals and groups:

Twenty five adult male albino rats with body weights of 180 ~ 200g were purchased from Cairo University animal house and acclimated for 1 week prior. Rats were housed in stainless steel wire cages and maintained at 24°C±2°C with a 12h light: Dark cycle and received standard chow diet and tap water ad libitum.

The rats were then divided into 3 groups, as follows:

1- Normal control rats (n=5) (Control) received an equal volume of vehicle (0.01 M citrate buffer, pH 4.5).

2- STZ-induced diabetic rats (DM) (n=10).

3- STZ-induced diabetic rats treated with ginseng (DM+ginseng) (n: 10) (GINSING Sigma) in a dose of 200mg/Kg given daily 2 weeks after induction of diabetes for 4 weeks. All experimental procedures were approved by the Institutional Animal Care Ethics Committee of Faculty of Medicine Cairo University and in accordance to the 8th edition, National Academies Press.

This study was conducted at Cairo University animal house from Nov. 2023 – April 2024.

Induction of diabetes:

By a single intraperitoneal injection of 60mg/kg streptozotocin (STZ). One week after these injections, blood samples were obtained from the tail vein. Rats with a blood glucose level greater than 300mg/dL were considered to be diabetic.

Experimental measurements:

During the experiment blood glucose level was measured after 10 days of STZ injection to check for diabetes and rats below 250mg/dl were excluded. At the end of the experiment 24h urine was collected then animals were sacrificed and fasting blood samples were withdrawn from abdominal aorta and hearts were removed from all groups of animals then cut vertically as two halves. One half was put in tubes containing formalin (10%) for pathological examination. The other half was stored at –80°C.

Blood glucose:

Blood glucose was testedusing glucose test strips and glucometer (On-Call Plus, Acon Laboratories Inc, USA).

At the end of the experimental period, animals were euthanized by halothane anesthesia, terminal blood samples were collected. Serum was then separated by centrifugation at 4,000 rpm (3,436× g) for 5 minutes and stored at −20°C until analysis.

Serum of insulin:serum insulin levels were determined using rat Insulin ELISA kit from Mercodia AB, (Sweden) as per manufacturer's instructions.

Plasma fatty acids: Plasma fatty acids concentration concentrations were measured by enzymatic methods (Roche).

Serum Glutathione (GSH): GSH was assayed by ELISA, results were displayed in mg/dl.

Glutathione peroxidase (GPX): GPX activity was determined using a GPX assay kit (Randox Lab., Ltd., UK, results were assessed in mg/dl.

Serum level of IL-6: IL-6 level was measured by means of enzyme-linked Immunosorbent Assay (ELISA) technique, using Rat ELISA kit (R and D Systems, USA), results were stated as pg/ml.

Serum level of cardiac enzymes: Serum CK-MB and troponin I were determined using specific ELISA kits supplied by EIAab (Wuhan, China).

Assessment of serum Beta actinin: Using specific ELISA kits supplied by EIAab (Wuhan, China).

Assessment of serum tissue Caspase 3: Using specific ELISA kits supplied by EIAab (Wuhan, China).

Histopathology:

Cardiactissues were fixed in 10% neutralized formaldehyde and embedded in paraffin prior to preparing 4-μm sections. The sections were stained with periodic acid-Schiff reagent (Sigma, St. Louis, MO, USA) and counterstained with hematoxylin.

Statistical methods:

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 22. Data was summarized using mean ± standard deviation. Comparisons between quantitative variables were done using one way analysis of variance (ANOVA) with post hoc Tukey test. *p*-values less than 0.05 were considered as statistically significant.

Results

Results showed significant deterioration in diabetic non treated group of animals as was seen in elevated levels of glucose and fatty acids and decreased serum levels of insulin. On administration of ginseng there was improvement in all diabetic

parameters depicted by decreased glucose and fatty acid levels and increased serum levels of insulin (Table 1).

Diabetes increased oxidative stress and reduced anti stress factors as was observed in elevated levels of Glutathione peroxidase(GPX) and reduced Glutathione (GSH) compared to normal control group. Ginseng is considered as antioxidant as this was confirmed by our results via decreased serum levels of Glutathione peroxidase (GPX) and increased serum levels of Reduced Glutathione (GSH) (Table 2).

Our results depicted deterioration of cardiac functions in diabetic groups as was observed by increased serum levels of cardiac enzymes (CK-MP and Troponin) compared to normal control group. Beta catenin as an indicator of cardiac muscle deterioration was elevated in diabetic group in comparison to normal control group (Table 3).

On the other hand Ginseng administration to diabetic group, there was significant improvement as was observed by significant reduction of serum levels of cardiac enzymes (CK-MP and Troponin), moreover, significant decrease in Beta catenin compared to diabetic group (Table 3).

In this study we tried to search for the mechanism by which diabetes deteriorates the cardiac function, results showed significant increase in serum levels of IL-6 and Caspase 3. Ginseng is known to be as anti-oxidant , as well as it is considered as anti-inflammatory and antiapoptotic as shown by the reduced serum levels of IL-6 and caspase 3 (Table 4).

Histological results delectated deterioration of cardiac tissue as shown by cardiac fibers necrosis and edema. Administration of ginseng improved the cardiac conditions as it was noticed by reduced oedema and reduced necrosis. (Fig. 1-A,B,C,D,E,F).

Table (1): Comparison mean values of serum level of insulin, glucose, free fatty acids in different groups.

Parameters / Groups	Insulin IU/ml	Glucose m Mole/ml	Free Fatty acids (mMole/ml)
Control	18.2	4.7	50.9
Diabetic (DM)	7.7*	14.35*	138.3*@
DM+Ginsing	11.6*@	7.5*@	81.8*@

* : Significant $p<0.05$ in comparison to control group.
@:Significant $p<0.05$ in comparison to group DM group.

Table (2): Comparison mean values of serum level of GPX, beta catenin and reduced gluthathione in different groups.

Parameters / Groups	Glutathione peroxidase (GPX) (mmol/l)	Reduced Glutathione (GSH) (mmol/l)
Control	53.9	70.7
Diabetic (DM)	117.8*	21.9*
DM+Ginsing	97.6*@	51*@

* : Significant $p<0.05$ in comparison to control group.
@:Significant $p<0.05$ in comparison to group DM group.

Table (3): Comparison mean values of serum level of CK-MP and Troponin in different groups.

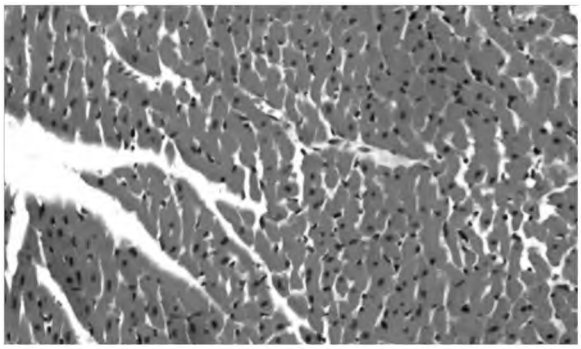
Parameters / Groups	CK-MP (ng/ml)	Troponin (ng/ml)	Beta catenin (mmol/l)
Control group	123.0±7.4 ng/ml	0.5±0 ng/ml	1±0
Diabetic group	260.32±46.35 ng/ml*	2.09±0.21 ng/ml*	5.91±1.57*
Diabetic + ginseng	149.0±31.28 ng/ml*@	2.2±0.2 ng/ml*@	2.6±0.52*@

* : Significant $p<0.05$ in comparison to control group.
@:Significant $p<0.05$ in comparison to group DM group.

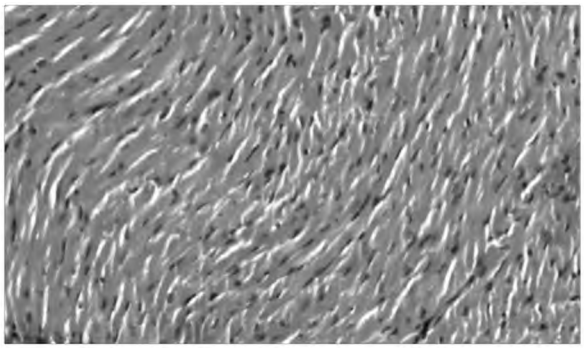
Table (4): Comparison mean values of serum level of IL-6 and Caspase 3 in different groups.

Parameters / Groups	IL-6 (ng/ml)	Caspase 3 (ng/ml)
Control group	17.6±4.9 ng/ml	2±0.5* ng/ml
Diabetic group	118.7±6.72* ng/ml	10.85±1.54* ng/ml
Diabetic + ginseng	50.2±21.83* ng/ml @	3±0.52*@

* : Significant $p<0.05$ in comparison to control group.
@:Significant $p<0.05$ in comparison to group DM group.



(1-A)



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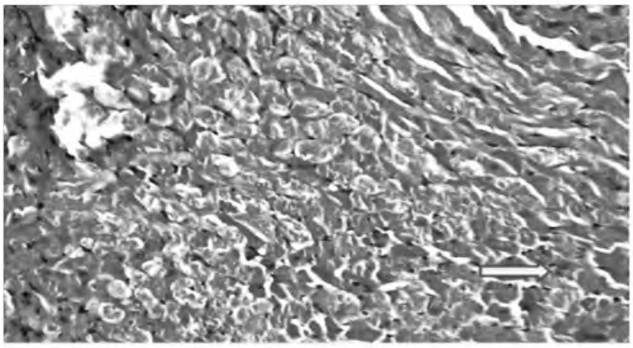

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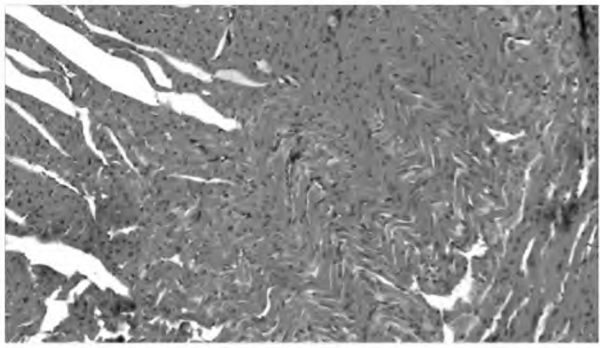

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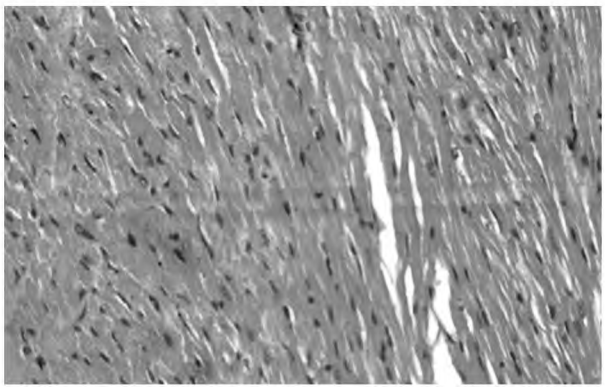

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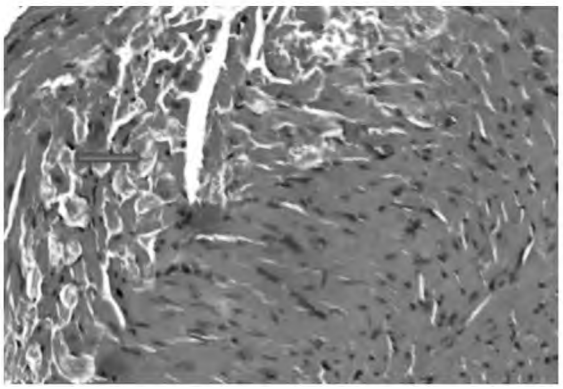

(1-F)

Fig. (1-A): Heart of a rat of control normal group (N): Showing normal histological structure of cardiac muscle fibers with normal cardiac striations (H&E, X200).

Fig. (1-B): Heart of a rat of control normal group (N): Showing normal histological structure of cardiac muscle fibers with normal cardiac striations (H&E, X400).

Fig. (1-C): Heart of a rat of diabetic group (D) showing interstitial edema in between cardiac muscle fibers , some myocytes appeared necrotic with loss of striations of sarcoplasm and karyolysis of the nuclei (yellow arrow) (H&E, 200).

Fig. (1-D): Heart of a rat of diabetic group (DM) showing interstitial edema in between cardiac muscle fibers, some myocytes appeared necrotic with loss of striations of sarcoplasm and karyolysis of the nuclei) (H&E, 200).

Fig. (1-E): Heart of a rat of group treated by ginsing (DM+Ginsing) showing moderate myocytes necrosis and intestinal edema in between muscle fibers (arrow) (H&E, 400).

Fig. (1-F): Heart of a rat of group treated with ginseng (DM+Ginsing) depicating deeply eosinophilic sarcoplasm of some cardiomyocytes, with loss of striations. Karyolysis of the nuclei, and interstitial edema in between muscle fibers are seen (H&E, 400).

Discussion

The study demonstrates the efficacy of ginseng-related therapy in improvement of glucose, insulin levels and fatty acid levels in rats with STZ induced diabetes. Our results are in accordance with reports elsewhere indicate that supplementation with Korean red ginseng rootlet preparation maintained good glycemic control and improved plasma glucose and insulin levels **[11]**.

The anti-diabetic effect of ginseng cannot be elucidated but it could be attributed to the possible mechanisms include modulations of, insulin production and secretion; glucose metabolism; glucose uptake; and inflammatory pathway **[12]**.

Further, ginsenosides are shown to activate AMP-activated protein kinase (AMPK) pathway. It has been suggested that ginsenosides may decrease the ATP biosynthesis, resulting in a change in the AMP: ATP ratio, which might further activate the AMPK pathway. Activation of AMPK pathway has been proposed as the mechanism for the suppression of hepatic gluconeogenesis and steatosis **[13]**. Ginseng is used previously as cardioprotective as it was found that rats treated with ginseng (250 or 500 mg/kg) inhibited the myocardial infarction after acute myocardial ischemia reperfusion injury **[13]** and isoproterenol-induced cardiac injury in rats **[15]**. Moreover, it was mentioned that ginseng (400 mg/kg) may enhance cardiac performance through an increase in the expression of PPARδ and without altering the heart rate in normal rats **[16]**. In the present study Ginseng improves cardiac functions in diabetic group administratedginseng and this was in agree with **[17]** as mentioned that cardiac performance in diabetic rats was also improved by repeated oral intake of ginseng at 150 mg/kg/day for one week and this used dose is markedly lower than used in previous reports for cardiac diseases. To the extent of our knowledge oxidative stress may play a key role in the pathogenesis and development of diabetes **[18]**. Further studies have provided evidence that oxidative stress has a relationship with diabetic complications such as DN **[19]**. In the present study ginseng treated rats improved oxidative stress. Ginsenosides compound decreased the oxidative stress marker MDA, and enhanced SOD in animal models of Diabetic nephropathy **[20]**. Low-grade inflammation is a key cause of diabetes **[21]**. As depicted from our results ginseng supplementation improves the inflammatory condition. Ginseng upregulated GPR120 expression in RAW264.7 macrophages, which lowered the level of iNOS and COX-2 expression to provide an anti-inflammatory effect; thus, it may be a viable solution to relieve inflammation and improve glucose metabolism **[22]**. Moreover, intraperitoneal injection of ginseng decreased the levels of pro-inflammatory cytokines, including TNF-α, IL-6 and or IL-1β and NF-κB pathway molecules (p-IKK and p-IκBα) in an animal experiment **[23]**. In the present results diabetic rats showed deterioration of cardiac parameters (cardiac enzymes and Beta catenin) while these parameters were improved on administration of ginseng. The present results showing ginseng cardio protection could be explained by reduction of myocardial apoptosis seen via reduced levels of caspase 3. Moreover, in vitro experiments showed that Rh2 activated PPARδ in cardiomyocytes cultured in high glucose, which inhibited the expression of STAT3, reduced cardiac fibrosis, and protected against diabetic cardiomyopathy **[24]**. Ginsenoside decreased the percentage of apoptotic myocardial cells and increased the parameters of cardiac function; it prevented myocardial lesions and myocardial collagen volume fraction. In rat models of diabetes, the mechanism through which ginsenoside Rg1 ameliorates diabetic cardiomyopathy is the inhibition of ER stress-induced apoptosis **[25]**. In conclusion, ginseng can improve diabetic cardiomyopathy via its ant inflammatory and antiapoptotic actions. Further investigation are needed to be done to consider ginseng one of anti-diabetic drug supplement to ameliorate diabetes and reduced its complications.

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تأثير الجنسنج على الستربتوزوتوسين المحدث لاعتلال عضلة القلب السكرى فى ذكور الجرذان

مقدمة: مرض السكرى أحد أكثر الاضطرابات الأيضية شيوعًا فى جميع أنحاء العالم. يعتبر اعتلال عضلة القلب السكرى من المضاعفات الكبيرة المرتبطة بمرض السكرى والتى يمكن أن تؤثر على المرضى مما يسبب الوفيات والمراضة. الجينسنغ هو أحد المنتجات الطبيعية المستخدمة فى الطب التكميلى.

الهدف من الدراسة: هدفت الدراسة الحالية إلى معرفة تأثير الجنسنج على قلب مريض السكر والآليات الكامنة وراءه.

المواد والطرق: تم إدخال ذكور الجرذان البيضاء البالغة بأوزان جسم تتراوح بين ١٨٠ إلى ٢٠٠ جرام فى الدراسة؛ وتم تقسيمهم إلى: مجموعة السيطرة، مجموعة مرضى السكرى، مجموعة مرضى السكرى + الجنسنج. فى نهاية الدراسة تم جمع عينات الدم لتقدير نسبة الجلوكوز فى الدم، الأنسولين، الأحماض الدهنية، الإنزيمات القلبية، بيتا كاتينين، IL–٦، كاسبيز ٣، الجلوتاثيون بيروكسيديز والجلوتاثيون المختزل. تم جمع أنسجة القلب للتحليل النسيجى.

النتائج: أظهرت النتائج تدهوراً فى جميع المؤشرات في مجموعة مرضى السكرى بينما تحسنت جميع المؤشرات عند تناول الجينسنغ.

الاستنتاج: الجينسنغ يحسن اعتلال عضلة القلب السكرى ويمنع المزيد من تدهور القلب.