Received: 11 April 2022, Accepted: 5 May 2022

Original Research

Journal of Advanced Veterinary Research
(2022) Volume 12, Issue 3, 194-204

Molecular and Immunohistochemical Validation of *Panax ginseng* Ameliorating Effects on The Pancreatic β-cell Activity and Its Implication on Some Metabolic Aspects in Alloxan- Induced Type 2 Diabetic Male Rats

Shereen B. Gad, Abdeldayem M. Zakaria, Sobhy A. Hedaya, Mona H. Hafez, Aya H. Rohiem*

Department of Physiology, Faculty of Veterinary Medicine, Alexandria University, Egypt

***Correspondence**
Aya H. Rohiem, Department of Physiology, Faculty of Veterinary Medicine, Alexandria University, Egypt.
E-mail: ayavet172@gmail.com

Abstract

The modulatory effects of *Panax ginseng* on the pancreatic β-cell activity, glucose metabolism and its hepatoprotective action in alloxan induced - type 2 diabetic male rats were studied for 2 months. We divided the rats randomly into six equal groups; control; diabetic (T2DM); ginseng, ginseng post T2DM induction; ginseng pre T2DM induction and ginseng pre and post T2DM induction. The serum level of glucose, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglycerides (TG), total protein, albumin, globulin, albumin- globulin ratio (A/G), total bilirubin, both direct and indirect bilirubins, gamma glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), cytochrome P450 (CYP450) and the activity of hepatic glucose -6- phosphatase (G6Pase) and also, the activity of the antioxidant markers [glutathione peroxidase (Gpx), catalase (CAT), superoxide dismutase (SOD), in addition; the level of lipid peroxidation product; malondialdehyde (MDA)] in both the serum and the liver were measured. The histological structure of both the pancreas and the liver and the expressions of both insulin receptors (IR), adenosine monophosphate kinase (AMPK) and the percentage of the positive area of insulin secretion in the islets of Langerhans using immunohistochemistry technique were also estimated. The results revealed that the previously mentioned parameters were significantly improved after administration of *Panax ginseng* to diabetic rats. In conclusion *Panax ginseng* administration could be ameliorate and protect the male rats against type 2 DM and could be able to decrease the intensity of damage caused in the pancreas after alloxan injection.

KEYWORDS
Panax ginseng, Male rats, Oxidative stress, Insulin receptors, Diabetes type 2, Immunohistochemistry, Gene expression.

INTRODUCTION

The pancreas has both endocrine and exocrine functions and performs an important role in glucose metabolism and digestion of food. The exocrine portion makes about 98% of the pancreatic tissue that is responsible for both digestive enzymes and pancreatic fluid production (Kallis and Westaby *et al.*, 2014). The endocrine pancreas is responsible for regulation of glucose, nutrient homeostasis and metabolism. It is composed of the islets of Langerhans, most of the cellular mass are called beta (β)- cells that are found centrally in the islet and enclosed by alpha (α)-cells, delta (δ)-cells, ε-cells and pancreatic polypeptide (PP) cells (Brass *et al.*, 2010).

β-cells secrete insulin that control the levels of blood glucose. Mazza and Maffucci, (2014) reported that β-cell dysfunction or death plays the main role not only in type 1 diabetes mellitus (T1DM) but also in type 2 diabetes mellitus T2DM. T2DM (non-insulin dependent DM) caused by loss of the β-cell mass and so, loss of its function (Li and Gong, 2015).

Ragheb and Medhat (2011) reported that type2 DM is considered as a chronic disease which is characterized by insulin resistance connected with obesity because of the liberation of free fatty acids and inflammatory cytokines from the adipose tissue. Moreover, T2DM is distinguished by decreased insulin sensitivity and impairment of β-cell function caused by acquired and genetic factors (Mazza and Maffucci, 2014).

Alloxan leads to serious damage of β-cells, reduction of insulin secretion and consequently leading to hyperglycemia so it can be used to induce diabetes mellitus on experimental animals (Soto *et al.*, 2010). Hyperglycemia induced by alloxan injection can produce ROS (Gad and El- Maddawy, 2014). ROS play a crucial effect in the induction and pathogenesis of diabetes (Soto *et al.*, 2010).

Oxidative stress (OS) is the exaggerated presence of oxidants related to antioxidants that act efficiently as oxidizing agents, which recognized as free radicals, involving reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Paiva *et al.*, 2017). Buresh and Berg (2015) found that there is a group of enzymes which provoke ROS and RNS, such as, nitric oxide synthase, NADPH oxidase and myeloperoxidase. Superoxide dismutase,

ISSN: 2090-6277/2090-6269/

glutathione peroxidase, glutathione reductase and catalase enzymes are involved in the process of cellular redox balance, that can transform superoxide radicals into peroxides and peroxides transformed into water and hydrogen; so, they can be used to evaluate oxidative stress (Silva *et al.*, 2011). Zhang *et al.* (2020) reported that OS exerts a critical action in the progression of all cases of diabetes mellitus, especially T2DM. Also, OS plays a main function in the pathogenesis of diabetic complications.

T2DM is related to elevated OS resulting from various abnormalities involving hyperglycemia, dyslipidemia, and inflammation (Bukhari *et al.*, 2015). Gerber and Rutter (2017) found that overstimulation of beta cells by free fatty acids (FFA) or chronic hyperglycemia impaired the secretion of insulin. Hyperglycemia and high FFA lead to massive aggregation of both reactive nitrogen species (RNS) and reactive oxygen species (ROS) that can impair β-cell function because of the decreased antioxidants [glutathione peroxidase (Gpx), catalase (CAT) and superoxide dismutase (SOD)] expression in β-cells (Zhang *et al.*, 2020). Diabetes in laboratory animals was also induced by administration of alloxan through destruction of beta cells via production of free radicals mainly the superoxides (Gad and El-Maddawy, 2014).

Ginseng is the most recognized plant that widely used in traditional medicine, ginseng is a perennial herb (Araliaceae family), a species related to the Panax genus (Chen *et al.*, 2019a). Panax is originated from the Greek word that means "all healing". *P. ginseng* was used many years ago as an herbal medicine in China and it was found to have a lot of pharmacological roles, such as antidiabetic, anticancer, hepatoprotective, neuroprotective and anti-ageing effects (Li and Gong, 2015). Ginseng contains many components, involving ginsenosides, polysaccharides, polyacetylenes, phenolics, and alkaloids (Li and Gong, 2015). Ginsenosides that also known as (saponins), are the most bioactive constituent of ginseng that mainly responsible for its anti-diabetic effect. About two hundred types of ginsenosides produced from ginseng and its heat-processed products (Chen *et al.* 2019b).

Fatmawati *et al.* (2014) proved that *P. ginseng* used in the prohibition and treatment of diabetes and prevents its expected complications (Lee and Rhee, 2017). Ginseng modifies the levels of blood glucose via enhancing β-cell action and improving insulin sensitivity (Chen *et al.*, 2019a).

P. ginseng can up-regulate insulin receptor type A and glucose transferase type 2 (GLUT2) in diabetic rats (Abdelazim *et al.*, 2019). Also, ginseng can improve the elevated glucose level in mice blood by stopping the absorption of glucose through the intestine and suppress the secretion of hepatic glucose-6-phosphatase (Yuan *et al.*, 2012). Glucose-6-phosphatase (G6Pase) is very important for glucose homeostasis because it is one of the main hepatic enzymes concerned with glycogenolysis and gluconeogenesis. Ginsenosides can activate adenosine monophosphate protein kinase (AMPK) (Yuan *et al.*, 2012). Na *et al.* (2018) demonstrated that there is an obvious relationship between AMPK action and DM incidence. AMPK activation can improve the glucose uptake by the cells through the inhibition of hepatic gluconeogenesis (Yuan *et al.*, 2012).

Panax ginseng can protect against oxidative stress through free radical scavenging. Ginseng can scavenge hydroxyl radical and prevents the deterioration of unsaturated fatty acids produced by lipid peroxidation (Nam *et al.* 2018a). *P. ginseng* can suppress the peroxidation of lipids through scavenging of superoxides and hydroxyl radicals and metal chelation. The antioxidant effect of *Panax ginseng* is resulted from its flavonol glycosides content, that act as superoxide scavenger and then inhibiting hydroxyl radical lipid peroxidation in the cellular membranes (El kiki and Galal, 2018).

Immunohistochemistry (IHC) is a method used to identify tissue or cellular antigens depending on antigen-antibody interactions, it can identify the site of antibody binding by labelling of the antibody. The methods used for immunohistochemical staining involving the enzyme-labeled (immunoperoxidase) and fluorophore-labeled (immunofluorescence) antibodies to recognize proteins and other particles in the cells. Immunoperoxidase methods are used mainly to extract extra information which is not available by H & E staining technique, light microscope or by electron-microscope (Kabiraj *et al.*, 2015).

The objective of this study was to determine the possible modulatory impact of *Panax ginseng* on the pancreatic β-cell activity and its hepatoprotective actions in type 2 diabetic rats induced by alloxan.

MATERIALS AND METHODS

Panax ginseng [Ginsana®, Egyptian International Pharmaceutical Industries Company, (EIPICO), Egypt]. It was prepared freshly by dissolving the content of the capsules in physiological saline (0.9 % NaCl) until full solubility (Gad El-Karim *et al.*, 2017).

Anhydrous Alloxan was purchased from Sigma-Aldrich Ltd., NewYork, USA, as pink odorless powder. It was prepared freshly by dissolving the powder in physiological saline (0.9 % NaCl) until full solubility was reached (Gad and El-Maddawy, 2014).

Experimental animals and protocol

Sixty apparently healthy 5 months old male albino rats weighing 200.0±10.0 g were purchased from the Faculty of Agriculture, Alexandria University, Egypt. This study was conducted for 2 months at Physiology Department, Faculty of Veterinary Medicine, Alexandria University. Rats were acclimatized to laboratory conditions (equal light and dark hours, room temperature: 25.0±2.0ºC and natural humidity: 60±2%) for two weeks before the experimental procedures with free access to water and standard diet (23% crude protein, 5% crude fat, 3.35% crude fiber and energy 3000 Kcal/ Kg) (Elfagr Company, Alexandria- Cairo Desert Road, Egypt). Rats were housed in wire cages with suitable dimensions (80 x 60 x 40 cm) (10 rats / cage) and were treated and handled humanely in acquiescence with the guidelines of the animal care approved by the local Ethical Committee of Alexandria University.

At first, we made a pilot test on 5 rats to ensure the effect of alloxan in induction of T2DM (the fasting glucose level was measured in the serum of each rat after 3 days of alloxan injection to confirm diabetes (it was higher than 200 mg/dl).

The rats were divided randomly into six equal groups (10 rats each); control group (G1): the rats in this group received daily 1 ml saline / kg B.W. orally by gastric gavage for 2 months, diabetic (T2DM) group (G2): received a single dose of 140 mg alloxan /kg B.W. intraperitoneally (I/P) (Rahman *et al.*, 2017), ginseng group (G3): received daily 200 mg *P. ginseng* /Kg B.W., orally by gastric gavage (Abdelazim *et al.*, 2019) for 2 months, ginseng post T2DM induction group (G4): received daily 200 mg *P. ginseng* /Kg B.W., orally by gastric gavage for 2 months after I/P injection with one dose of 140 mg alloxan/kg B.W. ginseng pre T2DM induction group (G5): received daily 200 mg *P. ginseng* /Kg B.W., orally by gastric gavage for 2 months followed by I/P injection with one dose of 140 mg alloxan /kg B.W., ginseng pre and post T2DM induction group (G6): received daily 200 mg *P. ginseng* /Kg B.W. orally by gastric gavage for one month then received one dose of 140 mg alloxan /kg B.W. I/P. followed by orally administration of *P. ginseng* with the same dose for another one month.

N.B. All rats that were subjected to intraperitoneal injection of alloxan received overnight 5% glucose solution by gastric gavage to avoid death caused by transient hypoglycemia induced by alloxan injection (Gad and El-Maddawy, 2014).

Blood sampling

At the end of the experiment, individual 8 hrs fasting blood samples were collected from all rats in the experimental groups for serum rapid glucose estimation and the other blood samples were taken from the 12 hours fasted rats. All the samples were obtained from retro-orbital veins in clean and dry test tubes. Once the 12 hrs fasting blood samples obtained, serum was separated immediately and kept at -20ºC for subsequent determination of the other biochemical parameters.

Tissue sampling

All the rats were euthanized and eviscerated quickly after collection of all blood samples. The liver and the pancreas were rapidly removed, blotted dry and grossly examined. A part of the liver from each rat was stored frozen at –20ºC until the time of homogenization for subsequent determination of the biochemical parameters. Another piece of the liver and the whole pancreas were kept in 10% neutral formalin for the histological study and the immunohistochemistry (IHC) analysis. Also, another part of each liver specimen was kept in mRNA Later solution and kept at –20ºC till analyzed via real time PCR (rt PCR) technique for estimation of the genes expression of both adenosine monophosphate kinase (AMPK) and insulin receptors (IR).

Biochemical parameters

The serum was used for spectrophotometric determination of glucose (Trinder, 1959), TC (Richmond, 1973), HDL (Lopez-Virella, 1977), both VLDL and LDL were calculated according to Friedwald, *et al.* (1972), TG (Tietz *et al.*, 1959), in addition to total protein (Tietz, 1994), both albumin and globulin (Doumas *et al.*, 1971), bilirubin (Tietz, 1995), the activities of both ALT and AST (Reitman and Frankel, 1957) GGT (Szasz, 1976). The cytochrome p450 activity in the serum was estimated as described by Chandrani (2003) using Monobind ELISA kits (Lake Forest, CA 92630, USA) with a sensitivity of 0.17 ng/ml. G6Pase activity in the liver was colorimetrically estimated using spectrophotometer according to Hass and Byrne (1960). All the kits purchased from Bio-Diagnostic Company, Egypt.

Lipid peroxidation and antioxidant markers

Malondialdehyde (MDA), glutathione peroxidase (Gpx), catalase (CAT) and superoxide dismutase (SOD) activity in both serum and the liver homogenate were measured colorimetrically according to the methods of Ohkawa *et al.* (1979), Paglia and Valentine (1967), Aebi (1984) and Nishikimi *et al.* (1972), respectively. All the kits purchased from Bio-Diagnostic Company, Egypt except the kit used for determination of SOD obtained from Sigma-Aldrich Ltd., NewYork, USA.

Molecular determinations (SYBR green real time PCR analysis)

Total RNA was extracted from the liver specimen, then kept in mRNA Later solution at –20ºC using RNeasy Mini Kit (Qiagen, gmbh, Germany) (Catalogue No.74104); following the instructions of manufacturer.

Primers for the experimental genes

The primers were prepared as the following: AMPKα1:5′-ATC-CGCAGAGAGATCCAGAA-3′ (McCrimmon *et al.*, 2006), IR: 5′- CGT CAT CAA TGG GCA GTT- 3′ and 5′- GTG ACT TAC AGA TGG TTG GG- 3′ (Al-Sultany *et al.*, 2018) and housekeeping gene ß-actin: 5′-TCCTCCTGAGCGCAAGTACTCT-3′ and 5′ GCTCAGTAACAGTC-CGCCTAGAA- 3′ (Banni *et al.*, 2010).

Immunohistochemistry (IHC) analysis

The percentage of the positive area of insulin secretion in the pancreatic beta cells was determined using Image Analysis Software, NIH, USA following the method determined by Abdo *et al.* (2014).

Histological examination

After fixation the remaining parts of the liver and the pancreatic specimens in neutral buffered formalin (10%) for twenty four hours, tissue samples were processed via the common technique of paraffin embedding. From the processed paraffin blocks, 5 µm thick pieces were taken, stained using hematoxylin and eosin (HE) (Bancroft and Layton, 2013) and examined using light microscope.

Analysis of the SYBR green real time (rt) -PCR

Amplification curves and cycle threshold values (Ct) were measured using the software of the strata gene MX3005P. To determine the alteration of the expression of each gene on the RNA of the various samples, the Ct of each specimen was measured relative to the control using the delta delta Ct "ΔΔCt" method stated by Yuan *et al.* (2006).
Whereas ΔΔCt = ΔCt reference – ΔCt target ΔCt target = Ct control – Ct treatment and ΔCt reference = Ct control- Ct treatment.

Statistical analysis

All values are explained as means ± with standard errors. Data collected were analyzed using Statistical Analysis System (SAS, 2004) via one way analysis of variance (ANOVA, one way), the differences and the significance among different groups were assessed by Duncan's multiple rang test (Duncan, 1955).

RESULTS

Biochemical parameters

Serum glucose, proteins, bilirubin, and lipid profiles

As revealed in Table1, the levels of glucose, total cholesterol, LDL, VLDL and TG in the serum increased significantly ($P < 0.05$) in diabetic group (G2) relative to all other groups (G1, G3, G4, G5 and G6). Meanwhile, they decreased significantly ($P < 0.05$) in ginseng group (G3) compared to the control (G1) and the other treated groups (G4, G5 and G6); except, TG exhibited a non-significant ($P < 0.05$) variation in both ginseng group (G3) and the control group (G1). The levels of both glucose and total cholesterol in the serum were significantly ($P < 0.05$) lower in both ginseng pre T2DM induction group (G5) and ginseng pre and post T2DM induction group (G6) relative to ginseng post T2DM induction group (G4). But, the levels of LDL, VLDL and TG in the serum had non-significant ($P < 0.05$) variations between ginseng pre T2DM

induction group (G5) and ginseng pre and post T2DM induction group (G6). Also, the serum levels of both VLDL and TG exhibited a non-significant (P < 0.05) variation between the control (G1) and ginseng pre and post T2DM induction group (G6).

The serum level of HDL (good cholesterol) increased significantly (P < 0.05) in ginseng group (G3) relative to all other groups (G1, G2, G4, G5 and G6). As well as it increased significantly (P < 0.05) in ginseng pre and post T2DM induction group (G6) compared to ginseng post T2DM induction group (G4). But it had a non-significant (P < 0.05) variation between the control (G1) and ginseng post T2DM induction group (G4). Also, it had a non-significant (P < 0.05) difference between diabetic group (G2) and ginseng post T2DM induction group (G4). In addition, it exhibited a non-significant (P < 0.05) variation between ginseng pre T2DM induction group (G5) and ginseng pre and post T2DM induction group (G6).

As noticed in Table 2, the serum level of total protein increased significantly (P < 0.05) in the treated groups (G 3, G4 and G6) compared to the diabetic group (G2). As well as serum level of albumin increased significantly (P < 0.05) in the treated groups (G 3, G4 and G6) compared to the diabetic group (G2). But it exhibited non-significant (P < 0.05) variations among the treated groups (G 3, G4 and G6) and the control group (G1). Also, serum level of total protein exhibited a non-significant (P < 0.05) variation among the control (G1), ginseng post T2DM induction group (G4) and ginseng pre and post T2DM induction group (G6). As well as, it had a non-significant (P < 0.05) difference between ginseng group (G3) and ginseng post T2DM induction group (G4) and a non-significant (P < 0.05) difference between the diabetic group (G2) and ginseng pre T2DM induction group (G5).

The serum globulin level increased significantly (P < 0.05) in the treated groups (G3 and G4) relative to the control (G1). On the other hand, it decreased significantly (P < 0.05) in ginseng pre and post T2DM induction group (G6) compared to ginseng post T2DM induction group (G4). But it exhibited a non-significant (P < 0.05) variation among the treated groups (G 3, G4 and G5). A/G increased significantly (P < 0.05 in the treated groups (G 3, G4 and G6) compared to the diabetic group (G2). But it exhibited a non-significant (P < 0.05) difference among the control (G1), ginseng group (G3) and ginseng pre and post T2DM induction group (G6). Also, it had a non-significant (P < 0.05) difference among ginseng group (G3), ginseng post T2DM induction group (G4) and ginseng pre and post T2DM induction group (G6). As well as, it had a non-significant (P < 0.05) variation between ginseng post T2DM induction group (G4) and ginseng pre T2DM induction group (G5). In addition, it exhibited a non-significant (P < 0.05) difference between the diabetic group (G2) and ginseng pre T2DM induction group (G5).

Data present in Table 2, showed that serum levels of total bilirubin, direct and indirect bilirubins increased significantly (P < 0.05) in diabetic group (G2) relative to the control (G1) and all the treated groups (G3, G4, G5 and G6). However, serum levels of both total and indirect bilirubins decreased significantly (P < 0.05) in ginseng group (G3) compared to both ginseng post T2DM induction group (G4) and ginseng pre T2DM induction group (G5). Furthermore, serum level of direct bilirubin decreased significantly (P < 0.05) in ginseng group (G3) relative to the other treated groups (G4, G5 and G6). But, it had a non-significant (P < 0.05) variation between the control (G1) and ginseng group (G3).

Serum levels of both total and indirect bilirubins exhibited

Table 1. Effect of administration of *Panax ginseng* for 2 months on the serum level of glucose, total cholesterol, HDL, LDL, VLDL and TG in alloxan - induced type 2 diabetic male rats.

Group	Parameters					
	Glucose (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	TG (mg/dl)
G1 (control)	89.00±1.30^{e}	47.67±0.88^{e}	19.00±0.58b^{c}	22.17±0.98^{d}	5.93±0.18^{d}	29.67±0.88^{de}
G2 (diabetic T2DM)	195.00±4.70^{a}	111.67±1.45^{a}	15.33±0.88^{d}	75.00±5.20^{a}	16.17±0.44^{a}	82.33±1.45^{a}
G3 (ginseng)	77.60±2.01^{f}	37.33±1.76^{f}	28.00±1.53^{a}	7.67±4.81^{e}	4.67±0.33^{e}	24.00±2.08^{e}
G4 (ginseng post T2DM induction)	122.40±2.11^{b}	86.00±2.31^{b}	16.00±0.58^{cd}	58.93±2.90^{b}	9.53±0.47^{b}	48.00±2.31^{b}
G5 (ginseng pre T2DM induction)	110.00±1.76^{c}	71.67±1.76^{c}	18.67±0.88^{bc}	44.87±1.27^{c}	7.53±0.24^{c}	38.67±0.88^{c}
G6 (ginseng pre and post T2DM induction)	102.20±0.58^{d}	59.00±2.08^{d}	19.33±0.88^{b}	35.20±1.42^{c}	6.70±0.59^{cd}	35.00±3.06^{cd}

Means ± SE. Means within one column of different letters differ significantly at (P < 0.05). N=10. *Panax ginseng* was administered orally by gastric gavage for 2 months (200 mg /Kg B.W.). Each rat received one dose of alloxan (140 mg /kg B.W) intraperitoneally (I/P). HDL: high-density lipoprotein, LDL: low-density Lipoprotein, VLDL: very low-density Lipoprotein, TG: triglycerides.

Table 2. Effect of administration of *Panax ginseng* for 2 months on the serum level of total Protein, albumin, globulin, A/G ratio, total bilirubin, direct bilirubin and indirect bilirubin, in alloxan - induced type 2 diabetic male rats.

Group	Parameters						
	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Indirect Bilirubin (mg/dl)
G1 (control)	7.67±0.12^{b}	4.03±0.09^{a}	3.50±0.06^{c}	1.15±0.04^{a}	0.16±0.02^{d}	0.07±0.01b^{c}	0.07±0.01d
G2 (diabetic T2DM)	6.63±0.15^{c}	2.73±0.09^{b}	3.83±0.09^{abc}	0.71±0.02^{d}	0.59±0.04^{a}	0.15±0.02^{a}	0.51±0.02^{a}
G3 (ginseng)	8.13±0.09^{a}	4.13±0.15^{a}	3.90±0.17^{ab}	1.07±0.08^{ab}	0.14±0.02^{d}	0.05±0.01^{c}	0.09±0.01^{d}
G4 (ginseng post T2DM induction)	7.87±0.03^{ab}	3.77±0.15^{a}	4.10±0.17^{a}	0.92±0.07^{bc}	0.26±0.02^{c}	0.09±0.00^{b}	0.17±0.02^{c}
G5 (ginseng pre T2DM induction)	6.77±0.09^{c}	2.93±0.09^{b}	3.83±0.03^{abc}	0.77±0.03^{cd}	0.39±0.02^{b}	0.14±0.01^{a}	0.27±0.02^{b}
G6 (ginseng pre and post T2DM induction)	7.57±0.12^{b}	3.80±0.12^{a}	3.63±0.09^{bc}	1.05±0.05^{ab}	0.20±0.01^{cd}	0.10±0.00^{b}	0.11±0.01^{d}

Means ± SE. Means within one column of different letters differ significantly at (P < 0.05). N=10. *Panax ginseng* was administered orally by gastric gavage for 2 months (200 mg /Kg B.W.). Each rat received one dose of alloxan (140 mg /kg B.W) intraperitoneally (I/P). A/G: Albumin/Globulin ratio.

non-significant (P < 0.05) variations among control group (G1), ginseng group (G3) and ginseng pre and post T2DM induction group (G6). Also, the serum levels of both total and direct bilirubins exhibited a non-significant (P < 0.05) variation between ginseng post T2DM induction group (G4) and ginseng pre and post T2DM induction group (G6).

Serum and hepatic enzymes activity

As shown in Table 3, the diabetic group (G2) showed a significant (P < 0.05) elevation in serum activities of ALT, AST, GGT, CYP450 and in hepatic activity of G6Pase compared to all other groups (G1, G3, G4, G5 and G6). However, enzyme activities decreased significantly (P < 0.05) in ginseng group (G3) relative to other treated groups (G4, G5 and G6).

Also, data present in Table 3, revealed that serum ALT and AST activities were decreased significantly (P< 0.05) in ginseng pre and post T2DM induction group (G6) compared to both ginseng post T2DM induction group (G4) and ginseng pre T2DM induction group (G5). Moreover, activities of both enzymes were significantly (P< 0.05) lower in ginseng pre T2DM induction group (G5) compared to ginseng post T2DM induction group (G4). Moreover, serum GGT activity decreased significantly (P< 0.05) in both ginseng post T2DM induction group (G4) and ginseng pre and post T2DM induction group (G6) relative to ginseng pre T2DM induction group (G5). But, it exhibited a non-significant (P< 0.05) variation between ginseng post T2DM induction group (G4) and ginseng pre and post T2DM induction group (G6).

Furthermore, the activities of both serum CYP450 and hepatic G6Pase decreased significantly (P< 0.05) in both ginseng pre T2DM induction group (G5) and ginseng pre and post T2DM induction group (G6) compared to ginseng post T2DM induction group (G4). But, they exhibited non-significant (P< 0.05) variations between ginseng pre T2DM induction group (G5) and ginseng pre and post T2DM induction group (G6) (Table3).

Lipid peroxidation and antioxidant markers

Serum glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activities and malondialdehyde (MDA) level

The obtained data in Table 4, revealed that serum level of MDA increased significantly (P < 0.05) in the diabetic group (G2) relative to all other groups (G1, G3, G4, G5 and G6). However, it decreased significantly (P < 0.05) in ginseng group (G3) relative to other treated groups (G4, G5 and G6). Moreover, it decreased significantly (P < 0.05) in ginseng pre and post T2DM induction group (G6) compared to both ginseng post T2DM induction group (G4) and ginseng pre T2DM induction group (G5). Furthermore, MDA level decreased significantly (P < 0.05) in ginseng pre T2DM induction group (G5) compared to ginseng post T2DM induction group (G4). But it exhibited a non-significant (P < 0.05) variation between the control (G1) and ginseng group (G3).

Also, data present in Table 4, showed that serum activities of Gpx, CAT and SOD increased significantly (P < 0.05) in ginseng group (G3) compared to all other groups (G1, G2, G4, G5 and G6). Moreover, serum activities of both Gpx and CAT were significantly (P < 0.05) higher in both ginseng pre T2DM induction group (G5) and ginseng pre and post T2DM induction group (G6) compared to ginseng post T2DM induction group (G4). But, they exhibited non-significant (P < 0.05) variations between pre T2DM induction group (G5) and ginseng pre and post T2DM induction group (G6).

Serum SOD activity increased significantly (P < 0.05) in ginseng pre and post T2DM induction group (G6) relative to both ginseng post T2DM induction group (G4) and ginseng pre T2DM induction group (G5).

Meanwhile, the activity of Gpx, CAT and SOD was significantly

Table 3. Effect of administration of *Panax ginseng* for 2 months on the serum activity of ALT, AST, GGT, CYP450 and the liver G6Pase in alloxan - induced type 2 diabetic male rats.

	Parameters				
Group	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	CYP450 (ng/ml)	G6Pase (nmol/min/ml)
G1 (control)	33.20±2.58^{e}	62.20±3.84^{e}	3.73±0.27^{d}	2.27±0.34^{d}	2.54±0.22^{d}
G2 (diabetic) T2DM	66.80±1.02^{a}	152.60±2.11^{a}	10.43±0.52^{a}	16.04±1.38^{a}	10.62±0.74^{a}
G3 (ginseng)	30.60±0.68^{e}	65.40±1.40^{e}	3.90±0.17^{d}	2.50±0.26^{d}	2.68±0.39^{d}
G4 (ginseng post T2DM induction)	61.60±2.01^{b}	107.20±1.16^{b}	5.80±0.17^{c}	7.76±0.39^{b}	6.68±0.42^{b}
G5 (ginseng pre T2DM induction)	46.80±1.07^{c}	94.00±0.45^{c}	6.67±0.24^{b}	5.64±0.15^{c}	4.88±0.33^{c}
G6 (ginseng pre and post T2DM induction)	40.80±0.37^{d}	74.40±1.36^{d}	5.07±0.09^{c}	4.56±0.05^{c}	4.00±0.07^{c}

Means ± SE. Means within one column of different letters differ significantly at (P < 0.05). N=10. *Panax ginseng* was administered orally by gastric gavage for 2 months (200 mg /Kg B.W.). Each rat received one dose of alloxan (140 mg /kg B.W) intraperitoneally (I/P). ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma glutamyl transferase. CYP450: cytochrome p450, G6Pase: Glucose-6-phosphatase.

Table 4. Effect of administration of *Panax ginseng* for 2 months on the serum activity of MDA, Gpx, CAT and SOD in alloxan - induced type 2 diabetic male rats

	Parameters			
Group	MDA (nmol/ml)	Gpx (U/L)	CAT serum (U/L)	SOD serum (U/ml)
G1 (control)	3.28±0.29^{e}	60.80±0.58^{b}	42.80±1.39^{b}	59.60±0.93^{b}
G2 (diabetic T2DM)	18.06±0.48^{a}	19.40±1.63^{e}	13.80±0.97^{e}	20.00±0.71^{e}
G3 (ginseng)	3.74±0.13^{e}	65.60±1.86^{a}	47.20±0.86^{a}	72.20±1.11^{a}
G4 (ginseng post T2DM induction)	14.18±0.24^{b}	31.00±0.71^{d}	26.00±1.76^{d}	34.60±0.93^{d}
G5 (ginseng pre T2DM induction)	11.16±0.36^{c}	41.20±1.02^{c}	32.80±0.86^{c}	36.40±0.51^{d}
G6 (ginseng pre and post T2DM induction)	8.50±0.54^{d}	43.60±0.51^{c}	36.00±1.30^{c}	45.00±0.84^{c}

Means ± SE. Means within one column of different letters differ significantly at (P < 0.05). N=10. *Panax ginseng* was administered orally by gastric gavage for 2 months (200 mg /Kg B.W.). Each rat received one dose of alloxan (140 mg /kg B.W) intraperitoneally (I/P). MDA: Malondialdehyde. Gpx: Glutathione peroxidase. CAT: Catalase. SOD: Superoxide dismutase.

(P < 0.05) lower in the serum of the diabetic group (G2) relative to the control group (G1) and all other groups (G3, G4, G5 and G6).

Hepatic glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activities, and malondialdehyde (MDA) level.

Data present in Table 5, revealed that hepatic MDA level increased significantly (P < 0.05) in the diabetic group (G2) relative to all other groups (G1, G3, G4, G5 and G6). Also, it increased significantly (P < 0.05) in the treated groups (G4, G5 and G6) compared to the control group (G1). However, it decreased significantly (P < 0.05) in ginseng group (G3) relative to the other treated groups (G4, G5 and G6). Moreover, hepatic MDA level decreased significantly (P < 0.05) in ginseng pre and post T2DM induction group (G6) compared to both ginseng post T2DM induction group (G4) and ginseng pre T2DM induction group (G5). Furthermore, it decreased significantly (P < 0.05) in ginseng pre T2DM induction group (G5) compared to ginseng post T2DM induction group (G4). But it showed a non-significant (P < 0.05) variation between the control (G1) and ginseng group (G3).

Also, Table 5, showed that hepatic activities of Gpx, CAT and SOD decreased significantly (P < 0.05) in the diabetic group (G2) relative to all other groups (G1, G3, G4, G5 and G6). Meanwhile, they increased significantly (P < 0.05) in ginseng group (G3) relative to other treated groups (G4, G5 and G6). Moreover, they increased significantly (P < 0.05) in ginseng pre and post T2DM induction group (G6) relative to both ginseng post T2DM induction group (G4) and ginseng pre T2DM induction group (G5). Furthermore, their activities were increased significantly (P < 0.05) in ginseng pre T2DM induction group (G5) compared to ginseng post T2DM induction group (G4). The hepatic activity of both CAT and SOD was significantly (P < 0.05) higher in ginseng group (G3) relative to control group (G1).

Real time PCR analysis

Data present in Figure 1, revealed that expressions of both hepatic AMPK and IR genes were significantly (P < 0.05) higher in ginseng group (G3) relative to control group (G1) and other treated groups (G4, G5 and G6). Moreover, all other treated groups (G4, G5 and G6) showed higher expressions of both hepatic AMPK and IR genes compared to control group (G1). Also, ginseng pre T2DM induction group (G5) revealed a significant (P < 0.05) elevation in the expression of hepatic IR gene compared to ginseng post T2DM induction group (G4). However, both hepatic AMPK and IR gene expressions decreased significantly (P < 0.05) in the diabetic group (G2) relative to all other groups (G1, G3, G4, G5 and G6).

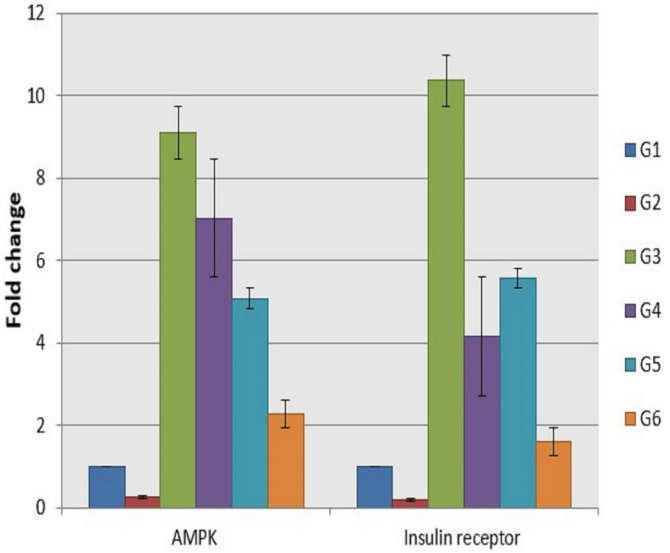

Fig. 1. Effect of administration of *Panax ginseng* for 2 months on the hepatic adenosine mono phosphate kinase (AMPK) and insulin receptors genes expression in alloxan - induced type 2 diabetic male rats. G1 (control), G2 (diabetic) T2DM, G3 (ginseng), G4 (ginseng post T2DM induction), G5 (ginseng pre T2DM induction), G6 (ginseng pre and post T2DM induction).

Immunohistochemistry of the pancreas (IHC) (insulin antibody immunostaining)

As illustrated in Figure 2, ginseng group (G3) revealed a significant (P < 0.05) increase in the percentage of positive area of insulin secretion in the pancreatic beta cells relative to the other treated groups (G 4, G5 and G6). However, the diabetic group (G2) revealed a significant (P < 0.05) decrease in the percentage of positive area relative to the control (G1) and the other treated groups (G3, G4, G5 and G6). Moreover, the percentage was decreased significantly (P < 0.05) in ginseng post T2DM induction group (G4) compared to ginseng pre T2DM induction group (G5) and ginseng pre and post T2DM induction group (G6). Also, it decreased significantly (P < 0.05) in ginseng pre T2DM induction group (G5) compared to ginseng pre and post T2DM induction group (G6). But it had a non-significant (P < 0.05) variation between the control (G1) and ginseng group (G3).

The Pancreas of both control group (G1) and ginseng group (G3) showed marked immunostaining of the insulin antibody within the β-cells, as shown in Figs. 3A & C, respectively. Meanwhile, the pancreas of diabetic group (G2) showed marked decrease of the expression of the insulin antibody within the β- cells (Fig. 3B). Conversely, the pancreas of both ginseng post T2DM induction group (G4) and ginseng pre T2DM induction group (G5) exhibited moderate increase of the insulin antibody expres-

Table 5. Effect of administration of *Panax ginseng* for 2 months on the hepatic activity of MDA, GPx, CAT and SOD in alloxan - induced type 2 diabetic male rats.

	Parameters			
Group	MDA (nmol/g)	Gpx (U /g)	CAT (U / g)	SOD (U/g)
G1 (control)	18.38±0.80^{e}	238.60±0.51^{a}	218.00±2.47^{b}	307.40±1.03^{b}
G2 (diabetic) T2DM	102.88±0.76^{a}	108.80±0.58^{f}	75.40±1.86^{f}	131.00±3.67^{f}
G3 (ginseng)	20.40±0.93^{e}	231.00±0.63^{b}	245.40±3.08^{a}	377.60±1.25^{a}
G4 (ginseng post T2DM induction)	72.80±1.88^{b}	159.40±0.87^{e}	139.20±1.59^{e}	180.00±0.71^{e}
G5 (ginseng pre T2DM induction)	57.20±2.63^{c}	217.20±0.86^{d}	169.20±2.42^{d}	223.80±0.58^{d}
G6 (ginseng pre and post T2DM induction)	42.80±0.97^{d}	220.40±0.81^{c}	190.20±6.37^{c}	233.40±1.44^{c}

Means ± SE. Means within one column of different letters differ significantly at (P < 0.05). N=10. *Panax ginseng* was administered orally by gastric gavage for 2 months (200 mg /Kg B.W.). Each rat received one dose of alloxan (140 mg /kg B.W) intraperitoneally (I/P). MDA: Malondialdehyde. Gpx: Glutathione peroxidase. CAT: Catalase. SOD: Superoxide dismutase.

sion within the β-cells (Figs. 3D & E, respectively). Moreover, the pancreas of ginseng pre and post T2DM induction group (G6) showing marked increase of the insulin antibody expression within the β- cells (Fig. 3F).

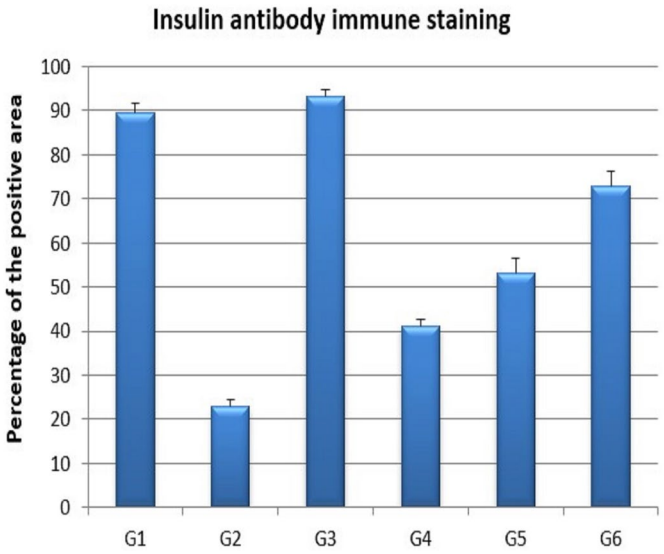

Fig. 2. Effect of administration of *Panax ginseng* for 2 months on the percentage of positive area of the insulin secretion in the beta cells (insulin antibody immunostaining) in alloxan - induced type 2 diabetic male rats. G1 (control), G2 (diabetic) T2DM, G3 (ginseng), G4 (ginseng post T2DM induction), G5 (ginseng pre T2DM induction), G6 (ginseng pre and post T2DM induction).

Histological examination

Histological examination of the pancreas

Effect of administration of Panax ginseng for 2 months on the diameter of the endocrine portion of the pancreas in alloxan - induced type 2 diabetic male rats

Data illustrated in Figure 4, revealed that the diameter of the endocrine portion of the pancreas decreased significantly ($P < 0.05$) in diabetic group (G2) relative to all other groups (G 1, G3, G4, G5 and G6). However, it was significantly ($P < 0.05$) higher in ginseng group (G3) compared to other treated groups (G4, G5 and G6). Moreover, it was significantly ($P < 0.05$) higher in ginseng pre and post T2DM induction group (G6) relative to both ginseng post T2DM induction group (G4) and ginseng pre T2DM induction group (G5). Furthermore, it increased significantly ($P < 0.05$) in ginseng pre T2DM induction group (G5) compared to ginseng post T2DM induction group (G4).

Effect of administration of Panax ginseng for 2 months on the pancreatic histology in alloxan - induced type 2 diabetic male rats

The pancreas of both control group (G1) and ginseng group (G3) showed normal endocrine structure (Figs. 5A and C, respectively). Meanwhile, the pancreas of diabetic group (G2) showed atrophy of the β- cells (Fig. 5B). Conversely, both ginseng post T2DM induction group (G4) and ginseng pre T2DM induction group (G5) revealed mild degenerative changes within the β cells (Figs. 5D and E, respectively). On the other hand, ginseng pre and post T2DM induction group (G6) showed only mild vacuolation of the β- cells (Fig. 5F).

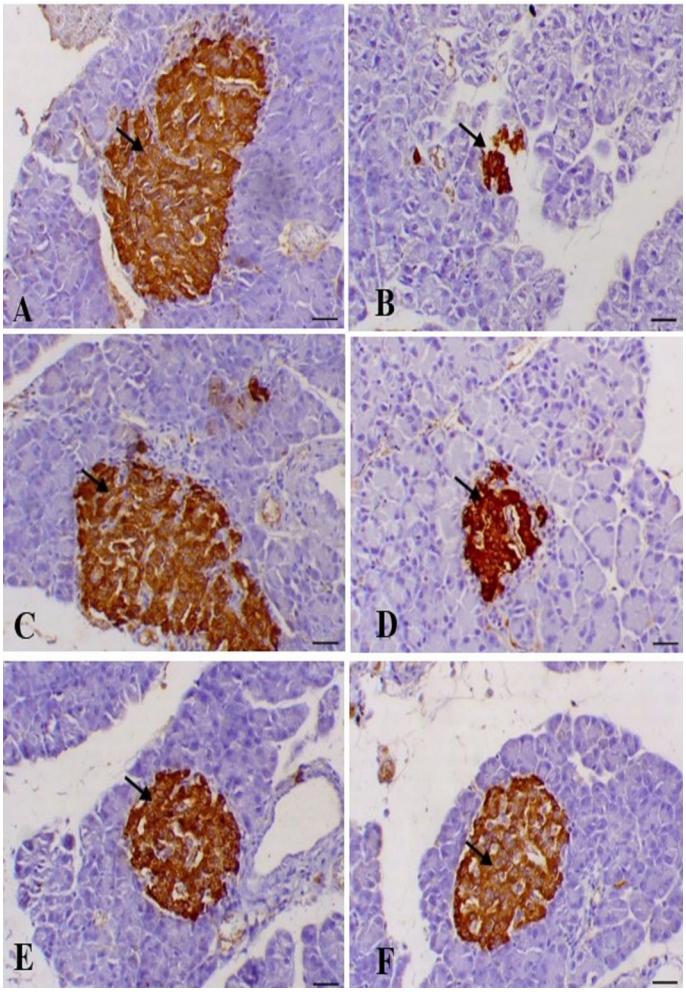

Fig. 3. Photomicrograph of rat pancreas. A) Control group (G1), showing marked immunostaining of the insulin antibody within the β- cells (arrow). B) Diabetic group (G2), showing very little expression of the insulin antibody within the β- cells (arrow indicates remnant of positive cells). C) Ginseng group (G3), showing marked expression of the insulin antibody within the β- cells (arrow). D) Ginseng post T2DM induction group (G4), showing moderate expression of the insulin antibody within the β- cells (arrow). E) Ginseng pre T2DM induction group (G5), showing an obvious expression of the insulin antibody within the β- cells (arrow). F) Ginseng pre and post T2DM induction group (G6), showing marked expression of the insulin antibody within the β-cells (arrow), X200.

Effects of administration of Panax ginseng for 2 months on the hepatic histology in alloxan - induced type 2 diabetic male rats

The liver of both control group (G1) and ginseng group (G3) showed normal hepatic cells around the central vein in the form of cords (Figs. 6A and C, respectively). Meanwhile, the liver of diabetic group (G2) revealed features of marked diffuse cytoplasmic vacuolation mostly of fatty and hydropic degenerative changes and few lymphocytic infiltrations (Fig. 6B). However, the liver of both ginseng post T2DM induction group (G4) and ginseng pre T2DM induction group (G5) showed marked decrease of the vacuolar degenerative changes within the periportal area (Figs. 6D and E, respectively). Meanwhile, the liver of ginseng pre and post T2DM induction group (G6) exhibited marked decrease in the hepatic vacuolation (Fig. 6F).

DISCUSSION

Pancreatic beta cell secretes insulin and control the level of plasma glucose. It was reported that β-cell dysfunction or death plays the main role not only in type 1 diabetes but also in type 2 diabetes (Mazza and Maffucci, 2014). T2DM, also known as non-insulin dependent diabetes mellitus, that attributed to loss the mass of β-cell and so, loss of its function (Li and Gong, 2015).

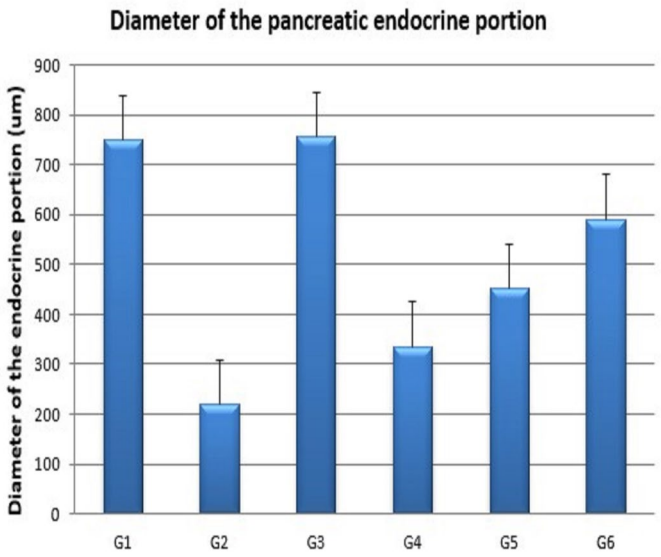

Fig. 4. Effect of administration of *Panax ginseng* for 2 months on the diameter of the endocrine portion of the pancreas (islets of Langerhans) in alloxan - induced type 2 diabetic male rats. G1 (control), G2 (diabetic) T2DM, G3 (ginseng), G4 (ginseng post T2DM induction), G5 (ginseng pre T2DM induction), G6 (ginseng pre and post T2DM induction).

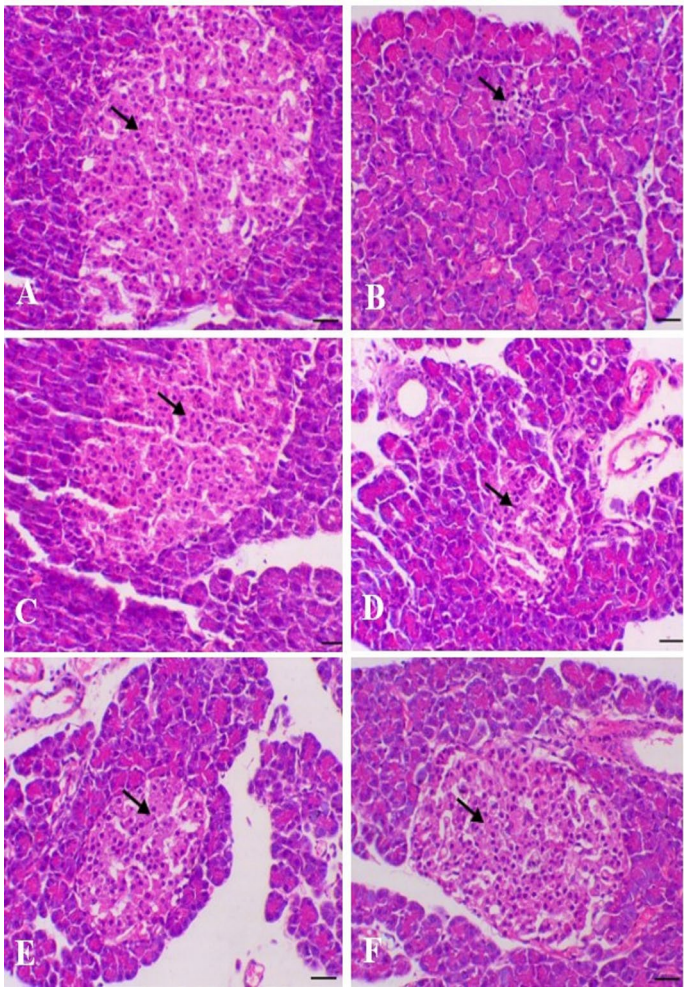

Fig. 5. Photomicrograph of rat pancreas. A) Control group (G1), showing normal endocrine structure (arrow indicates normal β- cells). B) Diabetic group (G2), showing atrophy in all the islets of Langerhans (arrow). C) Ginseng group (G3), showing normal endocrine structure (arrow). D) Ginseng post T2DM induction group (G4), showing mild degenerative changes within the β cells (arrow). E) Ginseng pre T2DM induction group (G5), showing mild degeneration of the pancreatic β- cells (arrow). F) Ginseng pre and post T2DM induction group (G6), showing mild vacuolation of the β- cells (arrow), H&E, X200.

The findings of the present study showed that treatment of the alloxan- induced type 2 diabetic male rats with *P. ginseng* for 2 months caused a significant decrease in the serum fasting glucose level and the other biochemical parameters, involving total cholesterol, LDL, VLDL, TG and increases the level of good cholesterol (HDL); these data agreed with the results of Kim *et al.* (2017) who found that ginseng decreased the level of blood glucose, cholesterol, LDL and total triglycerides; and agreed with the results of Abdelazim *et al.* (2019) who proved that ginseng can increase serum levelof HDL and reduce serum levels of total cholesterol (TC), TG and LDL.

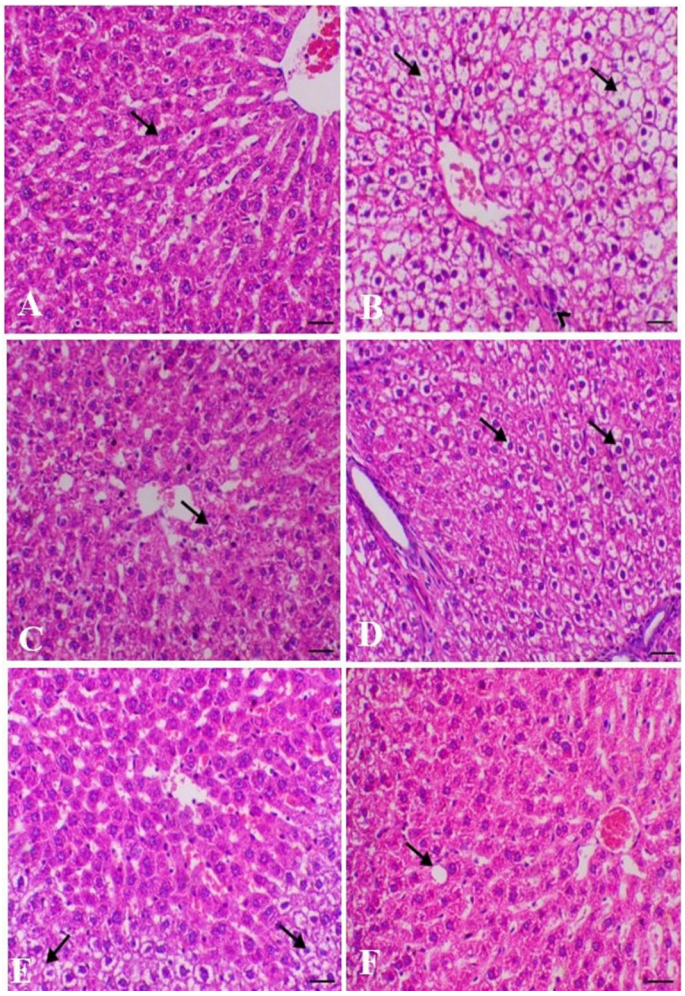

Fig. 6. Photomicrograph of rat liver. A) Control group (G1), showing normal hepatic cells around the central vein in the form of cords (arrow). B) Diabetic group (G2), showing marked diffuse cytoplasmic vacuolation mostly of fatty and hydropic degenerative changes (arrow) and few lymphocytic infiltrations (arrowhead). C) Ginseng group (G3), showing normal hepatocytes around the central vein in the form of cords with mild and granular cytoplasmic changes (arrow). D) Ginseng post T2DM induction group (G4), showing some hepatic cells with vacuolar degenerative changes (arrows). E) Ginseng pre T2DM induction group (G5), showing some hepatic cells with vacuolar degenerative changes at the peripheral area (arrows). F) Ginseng pre and post T2DM induction group (G6), showing apparently normal architecture with very little hepatic vacuolation. H&E, X200.

The modulatory effect of *P. ginseng* on serum glucose level could be attributed to its main component ginsenoside, which modifies the levels of blood glucose via enhancing β-cell action and improving insulin sensitivity (Chen *et al.*, 2019a). The lowering effect of *P. ginseng* on the serum cholesterol level may be attributed to its component ginsenoside mainly Rg1; which affects lipid metabolism and decreases TC level (Bai *et al.*, 2018).

Moreover, treatment of the diabetic male rats with *P. ginseng* caused a significant increase in insulin secretion evident by increasing the percentage of the positive area of insulin secretion and increasing the immunostaining of the insulin antibody within the β-cells using immunohistochemistry technique, this data agreed with the results of Wu *et al.* (2007) who proved that ginseng increased the cells of insulin production and improved pancreatic islet functions.

In the present study, treatment of diabetic male rats with *P. ginseng* for two months resulted in a significant elevation in the serum levels of total Protein, albumin, A/G ratio and decrease in

total, direct and indirect bilirubins levels, these findings agreed with the data of Shin *et al.* (2006) who found that *Panax ginseng* significantly ($P < 0.05$) enhanced the levels of TP in forced immobile mice. Also, these data agreed with the data of Nam *et al.* (2018b) who found that oral administration of ultrasonication-processed ginseng berry extract decreased the serum levels of bilirubin. Measurement of the protein levels in the blood is considered an important indicator for the nutritional status, liver disease and kidney disease and can indicated that *P. ginseng* act as a good preserver for the energy source. Serum albumin level is a good index for estimation of the liver function as it produced by liver (Shin *et al.*, 2006). Bilirubin is a toxic endogenous material, which is the end result of hemoglobin breakdown and acts as a diagnostic index of the hepatic and the blood disorders (Fevery, 2008).

Serum ALT, AST and GGT activities are considered as the major indicators of the organ dysfunction, the makers of both cellular damage and loss integritiy of the hepatic cellular membrane. Increases in the levels of the above-mentioned enzymes in diabetic rats may be due to oxidative stress evoked by hyperlipidemia (Uluisik and Keskin, 2016).

Treatment of diabetic male rats with *P. ginseng* for 2 months caused a significant ($P < 0.05$) improvement of type 2 diabetes. The hepatic improvement is evident by a significant reduction in serum levels of ALT, AST and GGT; these data agreed with the data of Uluisik and Keskin (2016) who found that red Korean ginseng significantly decreased serum activities of ALT, AST and GGT. Also, the obtained data agreed with the results of Kalkan *et al.* (2012) who found that *P. ginseng* significantly decreased serum ALT, AST and GGT activities. *P. ginseng* had a hepatoprotective effect which is mediated by its anti-apoptotic actions, the inhibition of inflammation and the suppression of c-Jun N-terminal protein kinase (JNK) activity (Bai *et al.*, 2018).

Moreover, serum cytochrome P450 activity in the serum decreased significantly ($P < 0.05$) in the treated groups relative to the diabetic one; this data agreed with the data of Lu *et al.* (2005) who proved that treatment with ginseng extract reduced cytochrome P450 content by 33% and they also found that I/P administration of ginseng Rb1 (GRb1) to rats resulted in reducing the liver cytochrome P-450 (CYP450) and the activity of Nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P-450 reductase compared to control rats.

Furthermore, hepatic G6Pase activity decreased significantly in treated groups compared to diabetic group; this finding agreed with the data of Jeon *et al.* (2013) who found that fermented ginseng extract (FGE) significantly ($P < 0.05$) decreased the expression of glucose-6-phosphotase (G6Pase) in hepatic tissues of db/db mice. G6Pase plays a crucial effect in glucose homeostasis as it is the major enzyme of both gluconeogenesis and glycogenolysis (Jeon *et al.*, 2013).

In addition, the hepatoprotective role of *P. ginseng* is obviously noticed as an improvement in the impaired hepatic histology, this effect agreed with the results of Kitts and Hu (2000) who proved that ginsenosides have a hepatoprotective effect via destroying reactive oxygen species and lipid peroxyl radicals.

Oxidative stress (OS) is the exaggerated presence of oxidants related to antioxidants that act efficiently as oxidizing agents, which recognized as free radicals, involving reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Paiva *et al.*, 2017). Buresh and Berg (2015) found that there is a group of enzymes which provoke ROS and RNS, such as, nitric oxide synthase, NADPH oxidase and myeloperoxidase. Superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase enzymes are involved in the process of cellular redox balance, that can transform superoxide radicals into peroxides and peroxides transformed into water and hydrogen; so, they can be used to evaluate oxidative stress (Silva *et al.*, 2011). Zhang *et al.* (2020) reported that OS exerts a critical action in the progression of all cases of diabetes mellitus, especially T2DM. Also, OS plays a main function in the pathogenesis of diabetic complications.

Treatment of diabetic male rats with *P. ginseng* for two months resulted in amelioration of the oxidative stress cleared by a significant increase in serum and hepatic activity of Gpx, CAT and SOD and decrease in MDA level, these data come in the same line with those of Anand *et al.* (2019). The present findings may be due to the free radical scavenging action of *P. ginseng*, it scavenges the hydroxyl radicals and save the unsaturated fatty acids from deterioration produced by lipid peroxidation (Nam *et al.* 2018a).

Treatment of the diabetic male rats with *P. ginseng* for 2 months significantly ($P < 0.05$) improved both AMKP and IR expression in the liver, these results agreed with the findings of Abdelazim *et al.* (2019) who found that AMPK gene expression level elevated in all rats treated with ginseng; also they found that ginseng significantly enhanced the expression of insulin receptors A (IRA) in diabetic rats treated with ginseng. Moreover, the obtained data agreed with the findings of Kho *et al.* (2016) who proved that ginseng up regulated the insulin receptors in the muscles of rat models of metabolic syndrome.

AMPK is considered as an important metabolic sensor which regulates the cellular energy homeostasis, it present in all eukaryotes as heterotrimeric complexes which consists of three major subunits; catalytic α, regulatory β and γ (Jeong *et al.*, 2014). AMPK can activate the catabolic process, such as glucose and fatty acids uptake and metabolism via both glycolysis and mitochondrial oxidation. As well as, AMPK can inhibit anabolic process, such as the hepatic manufacturing of glucose, glycogen and lipids. Activation of AMPK enhanced the insulin and Glucose transporter 4 (GLUT4) translocation in the muscles (Kim *et al.*, 2013). By inhibiting hepatic gluconeogenesis and enhancing glucose uptake by muscles and metabolism. So, there is an obvious relationship between AMPK action and DM incidence (Na *et al.*, 2018).

CONCLUSION

This study pointed to the capability of *P. ginseng* to ameliorate and protect male rats against DM type 2 through reduction the serum glucose, cholesterol and bilirubin levels, increasing the insulin and total proteins secretion, suppression of the oxidative stress, improving the hepatic enzymes level, modulation of both the AMPK and the insulin receptor expressions in the liver. Finally, we can conclude that the best group in achieving the aims of the study was the ginseng pre and post T2DM induction. So, it is recommended to use *P. ginseng* as a dietary supplement to avoid T2DM incidence to ameliorate the general health status and relieve some of its metabolic complications.

CONFLICT OF INTEREST

The author declares that no conflict of interests exists.

REFERENCES

Abdelazim, A. Khater, S. Ali, H., Shalaby, S., Afifi, M., Saddick, S., Alkaladi, A., Almaghrabi, O.A., 2019. *Panax ginseng* improves glucose metabolism in streptozotocin induced diabetic rats through 50 adenosine monophosphate kinase upregulation. Saudi Journal of Biological Sciences 26, 1436-1441.

Abdo, W., Ghattas, S., Sakai, H., Hirata, A., Yanai, T., 2014. Assessment of proliferative activity by proliferative cell nuclear antigen (PCNA) and anti bromodeoxyuridine (BrdU) immunolabeling in the tissues of Japanese eels (Anguilla japonica). Turkish Journal of Fisheries and Aquatic Sciences 14, 413-419.

Aebi, H., 1984. Catalase in vitro. Methods Enzymology 105, 121–126.

Al-Sultany, F.H., Al- Saadi, A.H., Al-Husainy, I.M., 2018. Evaluated the up-regulation in gene expression of hepatic insulin gene and hepatic insulin receptor gene in type 1 diabetic rats treated with cuscuta chinesis lam. Journal of University of Babylon for Pure and Applied Sciences 26,75-93.

Anand, S., Abul Hasan Ahmad, A.S., Telang, A.G., Dinesh Kumar, D., Pant, D., 2019. Ameliorating potential of *Panax ginseng* on oxidative stress following Subacute exposure to acetamiprid in rats. Journal of Animal Research 9, 339-344.

Bai, L., Gao, J., Wei, F., Zhao, J., Wang, D., Wei, J., 2018. Therapeutic poten-

tial of ginsenosides as an adjuvant treatment for diabetes. Frontiers in Pharmacology 9, 423- 436.

Bancroft, J.D., Layton, C., 2013. The Hematoxylin and Eosin. In: Theory & Practice of Histological Techniques. In Suvarna, S.K., Layton, C. & Bancroft, J.D. (Eds.). Seventh Ed. Churchill Livingstone of Elsevier. Philadelphia, USA, pp. 179-220.

Banni, M., Messaoudi, I., Said, L., El Heni, J., Kerkeni, A., Said, K., 2010. Metallothionein gene expression in liver of rats exposed to cadmium and supplemented with zinc and selenium. Archives of Environmental Contamination and Toxicology 59, 513-519.

Brass, B., Abelev, Z., Liao, E.P., Poretsky, L., 2010. Endocrine Pancreas. In. Principles of Diabetes Mellitus. Second Ed., Poretsky, L. Ed., Springer, NewYork, pp. 37-55.

Bukhari, S.A., Naqvi, S.A., Nagra, S.A., Anjum, F., Javed, S., Farooq, M., 2015. Assessing of oxidative stress related parameters in diabetes mellitus type 2: cause excessive damaging to DNA and enhanced homocysteine in diabetic patients. Pakistan Journal of Pharmaceutical Sciences 28, 483-491.

Buresh, R., Berg, K., 2015. A tutorial on oxidative stress and redox signaling with application to exercise and sedentariness. Sports Medicine Open 1, 3.

Chandrani, G., 2003. Drug metabolism and pharmacokinetics in drug discovery: A primer for bioanalytical chemists (Part I). Asian Journal of Drug Metabolism and Pharmacokinetics 3, 45-54.

Chen, W., Balan, P., Popovich, D.G., 2019a. Review of ginseng anti-diabetic studies. Molecules 24, 4501- 4517.

Chen, W., Balan, P., Popovich, D.G., 2019b. Chapter 6-Comparison of the ginsenoside composition of Asian ginseng (*Panax ginseng*) and American ginseng (Panax quinquefolius L.) and their transformation pathways. Studies in Natural Products Chemistry 63, 161–195.

Doumas, B.T., Waston, W.A., Biggs. H.G., 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clinical Chemistry Acta 31, 87- 96.

Duncan, D.B., 1955. Multiple range and multiple F. tests. Biometrics 11, 1-42.

El kiki, S.M., Galal, S.M., 2018. *Panax ginseng* extract modulates cerebral damage caused by gamma irradiation in male albino rats. Egyptian Journal of Radiation Sciences and Applications 31, 111–124.

Fatmawati, S., Ersam, T., Yu, H., Zhang, C., Jin, F., Shimizu, K. 2014. 20(S) Ginsenoside Rh2 as aldose reductase inhibitor from *Panax ginseng*. Bioorganic & Medicinal Chemistry Letters 24, 4407- 4409.

Fevery, J., 2008. Bilirubin in clinical practice: a review. Liver International 28, 592-605.

Friedwald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry 18, 499-502.

Gad El-Karim, D.R., Oda, S.S., Tohamy, H.G., Hashem, M.A., 2017. The Effect of Metformin and Ginseng on Alloxan-Induced Diabetic Rats: Hematological, Biochemical and Histopathological Studies. Alexandria Journal of Veterinary Sciences 55, 60-66

Gad, S.B., El-Maddawy, Z.K., 2014. Silymarin improves pancreatic endocrine function in rats. Pakistan Veterinary Journal 34, 214-218.

Gerber, P.A., Rutter, G.A., 2017. The role of oxidative stress and hypoxia in pancreatic βcell dysfunction in diabetes mellitus. Antioxidants & Redox Signaling 26, 501–518.

Hass, L. F., Byrne, W. L., 1960. The mechanism of glucose-6-phosphatase. Journal of the American Chemical Society 82, 947–954. Jeon, W.J., Oh, J.S., Park, M.S., Ji, G.E., 2013. Antihyperglycemic effect of fermented ginseng in type 2 diabetes mellitus mouse model. Phytotherapy Research 27, 166-172.

Jeong, K.J., Kim, G.W., Chung, S.H., 2014. AMP-activated protein kinase: An emerging target for ginseng. Journal of Ginseng Research 38, 83-88.

Kabiraj, A., Gupta, J., Khaitan, T., Bhattacharya, P.T., 2015. Principles and techniques of immunohistochemistry. International Journal of Biological and Medical Research 6, 5204-5210.

Kalkan, Y., Kapakin, K.A.T., Kara, A., Atabay, T., Karadeniz, A., Simsek, N., Karakus, E., Can, I., Yildirim, S., Oz-kanlar, S., Sengul, E., 2012. Protective effect of *Panax ginseng* against serum biochemical changes and apoptosisin kidney of rats treated with gentamicin sulphate. Journal of Molecular Histology 43, 603-613.

Kallis, Y.N., Westaby, D., 2014. Advanced Nutrition and Dietetics in Gastroenterology. In: Physiology and Function of the Pancreas. Chapter 1.6, Miranda Lomer (Ed.) First Ed. John Wiley & Sons, Ltd., pp. 33-35.

Kho, M.C., Lee, Y.J., Park, J.H., Kim, H.Y., Yoon, J.J., Ahn, Y.M., Tan, Park, M.C., Cha, J.D., Choi, K.M., Kang, D.G., Lee, H.S., 2016. Fermented red ginseng potentiates improvement of metabolic dysfunction in metabolic syndrome rat models. Nutrients 8, 369-383.

Kim, S.H., Hwang, J.T., Park, H.S., Kwon, D.Y., Kim, M.S., 2013. Capsaicin stimulates glucose uptake in C2C12 muscle cells via the reactive oxygen species (ROS)/AMPK/ p38 MAPK pathway. Biochemical and Biophysical Research Communications 439, 66-70.

Kim, J.M., Park, C.H., Park, S.K., Seung, T.W., Kang, J.Y., Ha, J.S., Lee, D.S., Lee, U., Kim, D.O., Heo, H.J., 2017. Ginsenoside Re ameliorates brain insulin resistance and cognitive dysfunction in high fat diet-induce C57BL/6 mice. Journal of Agricultural and Food Chemistry 65, 2719-2729.

Kitts D.D., Hu C., 2000. Efficacy and safety of ginseng. Public Health Nutrition 3, 473- 485.

Lee, S., Rhee, D.K., 2017. Effects of ginseng on stress-related depression, anxiety and the hypothalamic-pituitary-adrenal axis. Journal of Ginseng Research 41, 589-594.

Li, K.K., Gong, X.J., 2015. A review on the medicinal potential of *Panax ginseng* saponins in diabetes mellitus. The Royal Society of Chemistry 5, 47353- 47366.

Lopez-Virella, M.F., 1977. Cholesterol determination in high-density lipoproteins separated by three different methods Clin. Chem. 23, 882- 884.

Lu, R., Fan, H., Gao, J., Zeng, Y., 2005. Effect of ginseng extract and ginsenosides on activity of cytochrome P450. Asian Journal of Drug Metabolism and Pharmacokinetics 5, 17-24.

Mazza, S., Maffucci, T., 2014. Chapter Six-Autophagy and pancreatic β-cells. Vitamins and Hormones 95, 145- 164.

McCrimmon, R.J., Fan, X., Cheng, H., McNay, E., Chan, O., Shaw, M., Ding, Y., Zhu, W., Sherwin, R.S., 2006. Activation of AMP-activated protein kinase within the ventro medial hypothalamus amplifies counter regulatory hormone responses in rats with defective counter regulation. Diabetes 55, 1755-1760.

Na, R.S., Ma, C., Liu, Q.R., Wu, L.M., Zheng, X.L., Liu, Z.W., 2018. Itraconazole attenuates hepatic gluconeogenesis and promotes glucose uptake by regulating AMPK pathway. Experimental and Therapeutic Medicine 15, 2165–2171.

Nam, Y.J., Bae, J. Jeong, J.H., Ko, S.K., Sohn, U.D., 2018b. Protective effect of ultrasonication-processed ginseng berry extract on the D-galactosamine/ lipopolysaccharide-induced liver injury model in rats. Journal Ginseng Research 42, 540-548.

Nam, S. H., Choi, J. H., Song, Y.S., Lee, H.J., Hong, J.W., Kim, C.C., 2018a. Improved penetration of wild ginseng extracts into the skin using low temperature atmospheric pressure plasma. Plasma Sources Science and Technology 27, 1-9.

Nishikimi, M., Roa, N.A.,Yogi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazinemetho sulfate and molecular oxygen. Biochemical and Biophysical Research Communications 46, 849-854.

Ohkawa. H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 95, 351-358.

Paglia, D.E, Valentine, M.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine. 70, 158–169.

Paiva, L.A., Silva, L.S., de Souza, A.S., Cassino, P.C., 2017. Pulmonary oxidative stress in diabetic rats exposed to hyperoxia. Acta Cirúrgica Brasileira 32, 503-514.

Ragheb, R., Medhat, A.M., 2011. Mechanisms of fatty acid induced insulin resistance inmuscle and liver. Journal of Diabetes & Metabolism 2, 127- 131.

Rahman. S.S., Yasmin, N., Rahman, A.M.M., Akteruz Zaman, A., Rahman, M.H., Rouf, S.M.A., 2017. Evaluation and optimization of effective-dose of alloxan for inducing type-2 diabetes mellitus in Long Evans rats. Indian Journal of Pharmaceutical Education and Research 51, 661-666.

Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 28, 56- 63.

Richmond, N., 1973. Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clinical Chemistry 19, 1350- 1356.

SAS., 2004. (Statistical Analysis System) Statistical user's Guide. INT. Cary, NC. USA.

Shin, H.Y., Jeong, H.J., An, H.J., Hong, S.H., Um, J.Y., Shin, T.Y., Kwon, S.J., Jee, S.Y., Seo, B., Shin, S.S., Yang, D.C., Kim. H.M., 2006. The effect of *Panax ginseng* on forced immobility time & immune function in mice. Indian Journal of Medical Research 124, 199-206.

Silva, M., Lima, W.G., Silva, M.E., Pedrosa, M.L., 2011. Effect of streptozotocin on the glycemic and lipid profiles and oxidative stress in hamsters. Arquivos Brasileiros de Endocrinologia e Metabologia 55, 46-53.

Soto, C. J Pérez, V. García, E. Uría, M., Vadillo Raya, L., 2010. Effect of silymarin on kidneys of rats suffering from alloxan-induced diabetes mellitus. Phytomedicine 17,1090-1094.

Szasz, G., 1976. Reaction-rate method for gamma-glutamyl transferase activity in serum.Clinical Chemistry 22, 2051- 2055.

Tietz, N.W., 1994. Fundamentals of Clinical Chemistry: Second Ed. NW Tietz, editor, pp. 692.

Tietz N.W., 1995. Clinical Guide to Laboratory Tests. Third Ed. Philadephia: WB saunders,pp. 268-273.

Tietz, N.W. Boden, T., Stepleton, J.D., 1959. An improved method for the determinationof lipase in serum. American Journal of Clinical Pathology 31, 148-154.

Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with analternative oxygen acceptor. Annals of Clinical Biochemistry 6, 24-27.

Uluisik, D., Keskin, E., 2016. Hepatoprotective effects of ginseng in rats fed cholesterol rich diet. Acta Scientiae Veterinariae 44, 1-5.

Wu, Z., Luo, J.Z., Luo, L., 2007. American ginseng modulates pancreatic beta cellactivities. Chinese Medicine 2, 11-15.

Yuan H.D., Kim J.T., Kim S.H., Chung S.H., 2012. Ginseng and diabetes: the evidences from in vitro, animal and human studies. Journal of Ginseng Research 36, 27–39.

Yuan, J.S., Reed, A., Chen, F., Stewart, C.N., 2006. Statistical analysis of real time PCR data. BMC Bioinformatics 7, 85-96.

Zhang, P., Li, T., Wu, X., Nice, E.C., Huang, C., Zhang, Y., 2020. Oxidative stress and diabetes: antioxidative strategies. Frontiers of Medicine 14, 583–600.