Biochemistry Letters, 11(9) 2016, Pages: 90-101

Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Journal home page:

Effect of Panax ginseng on some changed biochemical parameters in alloxan-induced diabetic rats

Tayssir Ghoneim, Mohammed Elsaadani and Sherine Fawzy.

Biochemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt.

ARTICLE INFO

Article history:
Received
Accepted
Available online

Keywords:
.

ABSTRACT

Background: Panax ginseng root contains insulin like substances beside a mixture of saponin compounds responsible for its antioxidant activity. *Objectives:* The present work was carried out to investigate the effect of ginseng root extract (GRE) on some biochemical parameters in alloxan induced diabetic rats. *Methods*: Rats were divided into two main groups, the first group (control group) and the second group (diabetic group) in which the healthy rats were rendered diabetic, after 18 hours fasting, by a subcutaneous injection of a single dose of alloxan (120 mg/kg Body Weight "BW"). Three diabetic subgroups were treated orally with a daily dose of GRE (100 mg/kg BW) for 7, 14, and 21days, respectively. However, three subgroups were left diabetic without treatment for 7, 14, and 21 days. *Results:* Glucose 6-phosphate dehydrogenase (G6PDH) and 6-phospho-gluconate dehydrogenase (6PGDH) activities were significantly ($P<0.0001$) increased in the kidney extract of the diabetic rats after one week of diabetes induction. However, their activities in the liver extract were significantly decreased. Treatment of diabetic rats with GRE for 3 weeks showed restoration of the activities of G6PDH and 6PGDH in both kidney and liver. The abnormal elevations of kidney weight and the levels of glucose, glucose-6-phosphate, fructose and sorbitol in the kidney were markedly improved after treatment of diabetic rats with GRE. In addition, the level of serum glucose and potassium were significantly ($p<0.0001$) increased in all diabetic groups compared to control group. Conversaly, serum sodium level was decreased (hyponatreimia) in all diabetic groups. Treatment of diabetic rats with a single daily dose of GRE (100mg/BW) improved the abnormal changes of these parameters over all the periods of treatment especially after the 21 days of treatment.

INTRODUCTION

Diabetes causes a number of changes to the body's metabolism and blood circulation, which likely combine to produce excess reactive oxygen species. These changes damage the kidney's glomeruli which lead to the hallmark feature of albumin in the urine [1]. Accumulating evidences have indicated that Panax ginseng (Panax quinqrfolius), Family: *Araliaceae* (ginseng) possesses significant hypoglycemic activities [2-5]. Panax ginseng is a widely used traditional herb medicine [6, 7].

Junsang et al. [8] reported that venous administration of water soluble ginseng pharmacopuncture is the safe modality of treatment. Ginseng root contains a number of physiologically important substances including insulin like substances which have been reported to alleviate symptoms of a variety of degenerative diseases such as diabetes [9-11]. The most active component of ginseng root is a mixture of saponins called ginsenosides which have been reported to be responsible for antioxidant, antiperoxidant and organ protective actions of ginseng [12-16]. These actions of ginseng are linked to enhanced nitric oxide (NO) synthesis in endothelium of lung, heart and kidney [17]. Previous studies showed that ginseng promotes the synthesis of DNA, RNA and protein [18], beside decreases platelet adhesiveness in 66% hepactomized rats [19]. An alkaline fraction separated by ion exchange chromatography from water extract of Panax ginseng root stimulated the proliferation of baby hamster kidney –21 cells [20]. The study of Sohn et al.[21] demonstrated that proliferation of human renal cells carcinoma cells were inhibited by lipid soluble components of panax ginseng root. Also, it has been demonstrated that panax ginseng extract has an antinephrotoxic action against the nephrotoxicity of streptozotocin-induced diabetes in rats [22]. The renal complications of diabetes include a rapid initial hypertrophy in the short term and severe structural and pathophysiological damage in the long term [23]. Diabetic nephropathy (DN) is a progressive kidney disease caused by damage to the capillaries in the kidneys' glomeruli. It is characterized by nephritic syndrome and diffuse scarring of the glomeruli [24]. As diabetic nephropathy progresses, a structure in the glomeruli knowen as the glomerular filtration barrier (GFB) is increasingly damaged [1]. Therefore, the present study was undertaken to examine the impact glycemic control of GRE on attenuation of the progression of renal hypertrophy in alloxan–induced diabetic rats. This could be investigated through in vivo effect of the GRE on some biochemical parameters including the activity of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase in the kidney and liver. The kidney weight and the levels of serum sodium and potassium and levels of glucose, glucose-6-phosphate, sorbitol and fructose in kidney tissue were determined.

MATERIALS AND METHODS

Chemicals, reagents and medicinal plants

The kits of: Glucose oxidase, Sodium dependent β-galactosidase, Potassium dependent pyruvate kinase, Glucose-6-phosphate dehydrogenase assay, 6-phosphogluconate dehydrogenase assay, glucose-6-phosphate, Sorbitol and Fructose determination were purchased from Sigma(USA).Panax ginseng was obtained as brown powder from EPICO Co. for medicines, 10th of Ramadan, Egypt. Panax ginseng was freshly prepared by dissolving the powder in double distilled (ddH_2O) water.

Animals and experimental design

Sixty male sprague - dawley strain rats (weighing between 100-120 g) were obtained from the Animal House of Faculty of Medicine, Alexandria university, Alexandria, Egypt. The rats were housed in standard cages; the rats were given ad libitum access to food and water. After a period of one week of acclimation, animals were divided into two

main groups. The first group (6 rats) was used as control (C) and received double distilled water (ddH2O) as vehicle. The second group (54 rats) was rendered diabetic, after 18 hours fasting, by subcutaneous injection with a single dose of alloxan (120 mg/Kg BW) (25). Since the induction of diabetes was judged after 3 days by determination of glucose in blood samples collected from the tail vein of each rat. The rats which exhibited blood glucose levels higher than 300 mg/dl were selected. These diabetic rats (42 rats) were divided into six groups (7 rats each). The groups (1, 2 and 3) were left as diabetic rats without any treatment for 7, 14 and 21 days, respectively. While, groups (4), (5) and (6) were orally treated with a daily dose of 100mg GRE/Kg BW for 7, 14 and 21 days, respectively.

At the end of the experimental periods the diabetes induction, the rats had been fasted for 12h before cervical decapitation. Then the rats were cervical decapitated immediately after anesthesia using diethyl-ether as a volatile inhalational anesthetic agent. Blood samples were collected from each rat immediately after cervical decapitation and each sample was collected into clean tube. The blood samples were allowed to coagulate and then centrifuged at 3000 rpm for 5 min. The resulting supernatants, the sera, were carefully removed using a Pasteur pipette and kept at -20°C until used for the estimation of serum levels of glucose, sodium and potassium. Livers and kidneys were excised, weighed and washed using chilled saline solution. Kidney weight, and kidney levels of glucose, glucose-6-phosphate, sorbitol and fructose were determined, as well as the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in kidney and liver.

Preparation of tissue sample

The livers or kidneys were minced and homogenized in 0.32M sucrose /3mM $MgCl_2$ /2 mM EDTA /20 mM Tris HCl, pH 7.4 to obtain a 5% (w/v) extract. The homogenate was centrifuged at 1000 xg for 10 min. at 4 ^{o}C, the supernatant was further centrifuged at 100,000 xg for 30 min at 4 ^{o}C, and the supernatant (cytosolic fraction) was used for the assays of glucose-6-phosphate dehydrogenase (G6PDH) and 6-phospho-gluconate dehydrogenase (6PGDH) in the extract of the excised livers and kidneys. For kidney metabolites assay, 0.2 g of a fresh kidney was homogenized using 2 ml triethanolamine-HCl buffer (pH 7.5) then deproteinized using 2 ml perchloric acid followed by neutralization using 2 ml KOH. Then 6 ml of H_2O was added, mixed then centrifuged [26]. The separated supernatant was used for the determination of glucose, Glucose-6-Phosphate, sorbitol, and fructose.

Biochemical analysis

Serum glucose level was measured using glucose oxidase kit (27). Sodium serum level was determined via sodium dependent β-galactosidase activity using ortho-Nitrophenyl-β-galactoside (ONPG) as substrate (28). Serum potassium was determined via enzymatic determination of potassium dependent pyruvate kinase kit using phosphoenol pyruvate as a substrate, in a subsequent indicator reaction catalyzed by lactate dehydrogenase (LDH), the potassium dependent pyruvate is measured in terms of decrease of the NADH [29].

The activities of of glucose-6-phosphate dehydrogenase (G6PDH) and 6-phospho-gluconate dehydrogenase (6PGDH) in the tissue of livers and kidneys were estimated [30]. For the enzyme assay kits, the reduction of NADP was measured spectrophotometrically. A unit of enzyme activity is defined as 1µ mole of product formed per min at 25 ^{o}C. The assay of each

enzyme was run in each homogenate and the activities were expressed as unit /g fresh tissue. Glucose in kidney was determined using hexokinase and glucose-6-phosphate dehydrogenase [31]. Glucose-6-Phosphate was determined as it is oxidized to 6-phospho-gluconolactone which is converted spontaneously into 6-phosphogluconate [32]. D-Sorbitol assay kit was used for sorbitol determination in kidney, and Fructose assay kit was used for fructose estimation in kidney [33-34].

Statistical analysis

Statistical analyses were performed using the SPSS statistical software package (Statistical package for the Social Sciences, Salem, OR, USA). Data were presented as means with their standard errors. Normality and homogeneity of data were confirmed before ANOVA; differences among the control, diabetic and treated groups were assessed by one-way ANOVA followed by Scheffe test to analyze specific differences between means.

RESULTS

Diabetic rats left without treatment in the groups (1), (2) or (3) were characterized by a significant ($P<0.0001$) increase in their kidney weight compared to the control group (C) (Table 1). Table (1) illustrates also a highly significant ($P<0.0001$) increase in the glucose levels, observed in serum of diabetic rats in the same groups (1), (2) and (3) as compared with the control (C) group. Concerning serum sodium and potassium, hyponatremia and hyperkalemia were obviously observed in the three diabetic groups. The results showed also that there was a significant ($P<0001$) decrease in serum glucose level in treated diabetic groups (4), (5) and (6) which received daily treatment dose of GRE (100mg GRE/Kg BW) for 7, 14, 21 days respectively, as the glucose level was decreased to 23 %, 75 % and 77% as compared with the corresponding untreated diabetic groups (1),(2) and (3), respectively (Table 1).

Table (2) showed that the activities of both G6PDH and 6PGDH in the kidney of diabetic rats (group 1) significantly ($P<0.0001$) increased 100% above that of the control group. In the group (3), less pronounced changes in the activities of these enzymes were observed. Comparing the enzyme changes in the kidney with those occurring in the liver at the same periods, it is clearly revealed that in contrast to kidney, the two liver dehydrogenases significantly ($P <0.0001$) decreased (Table 2). The results of the present study showed that the levels of glucose, glucose-6-phosphate, fructose and sorbitol in kidney tissue were significantly ($P<0.0001$) increased over all the three periods of alloxan diabetes (Table 3).

DISCUSSION

The effect of different periods of diabetes alloxan-induced diabetes and treatment with ginseng on the kidney weight and serum glucose, sodium and potassium are shown in Table (1). Diabetic rats in the groups (1), (2) or (3) were characterized by a significant ($P<0.0001$) increase in their kidney weight compared to the control group (C). These results are in agreement with a previous study [35]. Diabetic renal changes are characterized by a progressive loss of renal function, oxidative stress, chronic inflammation, vascular remodeling, glomerulosclerosis, tubulointerstitial fibrosis and overt proteinuria [36]. It is clearly noticed that, the increase in kidney weight in the three diabetic groups paralleled with the increase in serum glucose level (Table 1). This is consistent with that previously reported [37]. The study of Paeivaensalo [38] have demonstrated that the diabetics had 4.8% larger kidneys ($P<0.039$). He also reported that the fasting blood glucose level is the most significant factor associated with enlarged kidney size. The decrease in serum sodium level (hyponatremia) and the increase in serum potassium level (hyperkalemia) were obviously observed in the three diabetic groups. These findings are correlated well with the work done by other investigators [39, 40]. The results in Table (1) showed that there

was a significant (P<0001) decrease in the serum glucose level in the diabetic groups (4), (5) and (6) which treated with GRE for 7, 14 and 21 days, respectively. Since glucose level was decreased to 23 %, 75 % and 77% as compared with the corresponding untreated diabetic groups (1), (2) and (3), respectively. This indicates that the hypoglycemic effect of ginseng exerts in a time dependant manner. Jenkins et al. [41] have demonstrated that the American ginseng attenuated postprandial glycemia in a time-dependant but not dose dependent manner in healthy individuals. The improvement in glucose level was associated with the improvement in serum Na+, K+ and kidney weight of treated groups (4), (5) and (6) (Table 1). This demonstrates that the glycemic control of ginseng may attenuate the increase in kidney weight, hyponatremia and hyperkalemia induced by alloxan. Nariman [42] demonstrated that ginseng intake to streptozotocin-diabetic rats significantly raised the serum sodium level and restored potassium concentrations to the normal values. These data supported the contention that ginseng potentially activates Na+ - K+ ATPase, as reported by Jin and Ski [43]. Mansour and Newairy [44] have indicated that oral administration of an aqueous extract of Balanites aegyptiaca fruits (mesocarp) caused significant increase in body weight and could normalize hyponatremia and hyperkalemia in streptozotocin –diabetic rats.

The activity of the enzymes catalyzing the oxidative segments of pentose phosphate pathway was increased in the rat kidney during the first 7 days after the induction of diabetes [37]. Thereafter, the enzyme activities returned towards control. The activities of both G6PDH and 6PGDH in the kidney of the diabetic rats (group 1) significantly (P<0.0001) increased 100% above that of the control group (Table 2). Interestingly, the increases in the activities of kidney G6PDH and 6PGDH are corresponded to the changes in two parameters. The first is: the highly significant lowering of serum Na^+ (Table1), this is in line with the known effect of sodium depletion on the activities of these enzymes. The second is: very marked increase in the kidney weight (Table 1). In the group (3), less pronounced changes in the activities of these enzymes were observed. It has been reported that the increase in kidney weight in diabetic rats is associated with an increase in the renal protein mass [45, 46] and this may be due to decrease in degradation of intracellular protein [47]. In addition, a decrease in protein turnover caused by inhibition of proteases contributes to renal hypertrophy [48].

The diabetic rats which treated with ginseng (groups 4, 5 and 6) exhibited a marked decrease in the activities of both kidney dehydrogenases (Table 2). Moreover, the treatment for 21 days showed normalization in the activities of the two enzymes. A comparison between the enzyme changes in the kidney with those occurring in the liver at the same periods, clearly revealed that in contrast to kidney, the two liver dehydrogenases significantly (P <0.0001) decreased (Table 2). It was well established that the diabetes causes a depression in the activity of G6PDH in the liver of diabetic rats [49]. Therefore, the early changes in the activities of the two dehydrogenases in the kidney of diabetic rats (Table 2) are specifically related to the diabetes and are not to short term toxic effect of alloxan itself. Table (2) also illustrates that ginseng intake caused a significant increase in the activities of liver dehydrogenases. The diabetic group (group 6) which received the extract for 21 days showed restoration in the activities of their liver dehydrogenases to the control value. Previous studies on the hypoglycemic effects of medicinal plants exert their hypoglycemic effects in association with a significant increase in the activity of liver G6PDH [50-52]. Previous study [53] reported that ginseng can improve hyperglycemia in mice by blocking intestinal glucose absorption and inhibiting hepatic glucose-6-phosphatase.

The levels of glucose, glucose-6-phosphate, fructose and sorbitol in kidney tissue were significantly (P<0.0001) increased over all the three periods of alloxan diabetes (Table 3). These substances are significant to pentose phosphate pathway, since they act as substrates or as acceptors of the NADPH.

Ludivigson and Sorenson [54] have pointed out that the rise in the content of sorbitol and fructose shows an increased activity of the sorbitol rout. This is in line with the known aldose reductase content of the kidney, its high Km for glucose and the high intracellular glucose concentration. Previous study on renal hypertrophy in experimental diabetes demonstrated that, the enzyme aldose reductase (AR) associated with polyol pathway, and oxidative stress is known to play an important role in the complications of diabetes [55]. This enzyme catalyzes the reduction of glucose to sorbitol which is subsequently converted to fructose by sorbitol dehydrogenase (SDH). Both aldose reductase (AR) and SDH constitute the sorbitol (polyol) pathway whose acceleration has been postulated to play a key role in the pathogenesis of diabetic complications [56]. The study of Hodgki et al. [57] showed that diabetic patients with nephropathy exhibit marked disturbances in the expression of enzymatic components of sorbitol pathway. Wallner et al. [58] have also reported that AR is up-regulated during hyperglycemia in streptozotocin-induced diabetes in mice. The increase in the content of kidney G6P is significant in relation to the rise in flux through the phosphate pentose pathway [37]. Meyer et al. [59] have concluded that in type 2 diabetes, both liver and kidney contribute to glucose over-production and that renal glucose uptake is markedly increased. The later may suppress renal free fatty acids uptake via glucose–fatty acid cycle and explain the accumulation of glycogen commonly found in diabetic kidney. Administration of diabetic rats with GRE for 21 days could restore the elevated levels of the four measured metabolites to the normal values (Table 3). Ginsenosides, the most active component of GRE, have been found to inhibit glucose uptake in primary cultured rabbit renal proximal tubular cells [60].

Generally, the present results clearly demonstrated the close relationship between the hyperglycemia and the change in the other parameters measured in the blood and kidney of diabetic rats. This was obviously observed in the diabetic group (1), i.e.: in the early stage of the alloxan–induced diabetes. Therefore, diabetic renal hypertrophy might be experimentally induced and established in the present study. Normalization of all parameters 21 days post-treatment with GRE suggests that the diabetic renal hypertrophy might be reversed. Previous study of Christoph et al. [47] showed that, the renal hypertrophy in streptozotocin–injected rats was prevented by insulin treatment. The presence of insulin –like substances in GRE as previously reported [61] may be a possible explanation for its capacity for attenuating the progression of diabetic renal hypertrophy. Yuan and Chung [62] have shown that the protective effect of GRE against STZ-induced pancreatic -cell damage by inhibiting the NF-_B activity. Finally, effects of GRE were reflected in the alleviation of the diabetes induced renal abnormalities of the studied biochemical parameters.

CONCLUSION

The glycemic control of ginseng may attenuate the progression of experimentally–induced diabetic renal hypertrophy as confirmed by decreasing kidney weight and inhibition of the activities of kidney G6PDH and 6PGDH In addition the improving hyponatremia and hyperkalemia beside the reduction of kidney content of glucose, glucose-6-phosphate, fructose and sorbitol. However, further biochemical and histological studies are required to provide additional evidence (s) for the efficiency of ginseng to ameliorate the complications associated to diabetes.

REFERENCES

(1) CAO, Z., and Cooper, M., 2011.Pathogenesis of diabetic nephropathy.*Journal of Diabetes Investigation*,2(4):243-247.

(2) Yuan, H.D., Kim, S.J., Chung, S.H.,2011. Beneficial effects of IH-901 on glucose and

lipid metabolisms via activating adenosine monophosphate-activated protein kinase and phosphatidylinositol-3 kinase pathways. *Metabolism*. 60:43–51.

(3) Lee, H.J., Lee, Y.H., Park, S.K., Kang, E.S., Kim, H.J., Lee, Y.C., Choi, C.S., Park, S.E., Ahn, C.W.,and Cha, B.S.,2009. Korean red ginseng (Panax ginseng) improves insulin sensitivity and attenuates the development of diabetes in Otsuka Long-Evans Tokushima fatty rats. *Metabolism.* 58:1170–1177.

(4) Luo, J.Z., Luo, L.,2009. Ginseng on hyperglycemia: effects and mechanisms. *Evid Based Complement Alternat Med*. 6:423–427.

(5) Vuksan, V., Sung, M.K., Sievenpiper, J.L., Stavro, P.M., Jenkins, A.L., Di Buono, M., Lee, K.S., Leiter, L.A., Nam, K.Y., and Arnason, J.T.,2008. Korean red ginseng (Panax ginseng) improves glucose and insulin regulation in well-controlled, type 2 diabetes: results of a randomized, double-blind, placebo-controlled study of efficacy and safety. *Nutr Metab Cardiovasc Dis*.18:46–56.

(6) Hofseth, L.J.,Wargovich, M.J.,2007.Inflamation,cancer,and targets of gihseng.*J Nutr*,Volume 137,pp.183S-185S.

(7) Lee, J.G., Lee Y.Y.,Kim S.Y., Pyo, J.S.,Yun-Choi H.S., and Park, J.H.,2009. Platlet antiaggregating activity of ginsenosides isolated from processed ginseng. *Pharmazie*,Volume64, 602-604.

(8)Junsang, Yu., Seungho, S., Kwangho, L., and kirok, K.,2015. Single- dose Toxicity of Water-soluble Ginseng Pharmacopuncture Injected Intramuscularly in Rats. *JPharmacopuncture*, June;18(2):76-85.

(9)Sotaniemi, E.A., Haapakoski, E., and Rauto, A., 1995. Ginseng therapy in non insulin dependent diabetic-patients effect on phycho-physical performance,glucose homeostasis , serum lipids, serum Aminoterminal propeptide concentration,and body weight .*Diabetes care*.,18 (10),1373-1375.

(10)Kimura, I., Nakashima, N., Sugihara, Y., Fu-Jun, C., Kimura, M., 1999. The antihyperglycemic blend effect of traditional Chinese medicine by akko-ka-ninjin to on alloxan and diabetic KK-CA (y) mice. *Phytother Res*.,13 (6),484-488.

(11)Vuksan, V., Sievenpiper, J.L., Koo, V.Y., Francis, T., Beljan-Zdravkovic, U., Xu, Z., Vidgen, E., 2000. American ginseng (panax quinquefolious) reduces postprandial glycemia in non diabetic subjects and subjects with type 2 diabetes mellitus .*Arch Intern Med.*,160 (7),1009-1013.

(12) Xie, Z. C., Qian, Z.K., Liu, Z.W., 1993. Effect of ginseng on antiperoxidate injury in myocardium and erythrocytes in streptozotocin –induced diabetic rats. Chung Kuo Chung His I Chieb Ho Tsa Chilh, 13,289-290.

(13) Okamura, N., Kobayashi, K., Akaike, A., et al., 1994. Protective effect of ginseng saponins against impaired brain growth in neonatal rats exposed to ethanol.*Biol Pharm Bull* (Japan).,17(2),270-274.

(14) Zhong, G., Jiang, Y., 1997. Calcium channel blockage and antifree-radical actions of ginsenosides. Chik Med J.110 (1),28-29.

(15) Maffei Facino, R., Carini,M., Aldini, G., et al.,1999.Panax ginseng admistration in the rat prevents myocardial ischemia –reperfusion damage induced by hyperbaric oxygen for an antioxidant intervension. *Planta Med* . (Germany).,65 (7),614-619.

(16) Kitts, D.D., Wijewickreme, A.N., Hu, C., 2000. Antioxidant properties of a North American ginseng extract. *Mol Cell Biochem*,203 (1-2),1-10.

(17) Gillis,C.N.,1997. Panax ginseng pharmacology; A nitric oxide link. Biochemical Pharmacology, 54(1),1-8.

(18) Chong, S.K. and Oberholzer, V.G., 1988.Ginseng is there a use in clinical medicine. *Post grad. Med.J.*, 64, 841-846.

(19) Cui, X., Sakaguchi, T., Shirai, Y., Hata Keyamak.,1999.Orally admistrated panax ginseng extract decreases platelet

adhesiveness in 66% hepactomized rats.,*Am J Chin Med*,27 (2), 251-256.

(20) Yagi, A., Akita, K., Ueda, T., Okamura, N., Itoh H.,1994.Effect of a peptide of panax ginseng on the proliferation of baby hamster kidney –21 cells. Planta Medica. 60, 171-174.

(21)Sohen,J;Lee,C.H.;Chung,D.J.;Park,S.H.;Kim,I;andHwang,W.I.,1998.Effect of petroleum ether extract of Panax ginseng roots on proliferation and cell cycle progression of human renal cell carcinoma cells.*Exp Mol Med*. 30,47-51.

(22) Nariman, K.B., 1997. Effect of panax ginseng on the nephrotoxicity of streptozotocin-induced experimental diabetes. *The Egyptian Journal of Biochemistry*.,15 (1,2), 29-52.

(23) Ku, D. D., Seller, B. M. and Meezan, E., 1986. Development of renal hypertrophy and increased renal Na-K –ATPase in streptozotocin diabetic rats .*Endocrinology*., 119,672-679.

(24) Maro-Fernandez C., Dominguez-Pimentel V.,and de Fuentes M.M., 2014. Diabetic kidney disease:from physiology to thearapeutics.
JPhysiol.(Lond.)592(Pt18):3997-4012.

(25) Dare, K. R. and, Katyare S.S.,2002. Effect of alloxan-induced diabetes on serum and cardiac butyrylcholinesterase in the rat. *J Endocrinol*., 175-241-50.

(26) Bergmeyer, H.U, 1974.Methods of enzymatic analysis. Academic Press,New York second edition.

(27) Trinder, P., 1969. Glucose GOD-PAD enzymatic colorimetric methods. Ann.*Clin. Biochem*, 6, 24.

(28) B Berry, M.N., Mazzachi, R.D., Pejakovic, M. and Peake, M.J., 1988. Enzymatic Determination of sodium in Serum *Clin.Chem*.34, 2295-2298.

(29) Berry, M.N., Mazzachi, R.D., Pejakovic, M. and Peake, M.J., 1989. Enzymatic Determination of Potassium in Serum.*Clin.Chem*.35 (5),817-820.

(30) Glock, G.E. and Mclean, P.,(1953).Further studies on the properties and assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver. Biochem.J., 55,400-408.

(31) Hans U.B., Eirch B., Feiix S. and Harald S.,1974.D-Glucose jn Methods of enzymatic analysis ,2nd ed.Academic Press,New York.pp.1197-1201.

(32) Gunter L., and Gerhard M.,1974. D-Glucose-6-phosphate in : Bergmeyer Methods of enzymatic analysis, 2nd ed. Academic Press,New York.pp.1238-1242.

(33)Hans-Otto B., 1986.D-Sorbitol in: Methods of enzymatic analysis, 3rd ed. Academic Press.New York. Pp, 356-362.

(34) Erich B., and Hans Ulrich B., 1974. D-Fructose in Methods of enzymatic analysis.2nd ed. Academic Press,New York press. pp: 1305-1307.

(35) Mohsen P., Ebrahim N., and Hamid Sh.,2014.Early Renal Histological Changes in Alloxan Induced Diabetic Rats.*IJMCM*,vol.3, No1,1-60.

(36) Parving H.H., Mauer M., Fioretto P., Rossing P., Ritz E., 2011. Diabetic nephropathy. In: Brenner B.M., editor. The kidney. WB Saunders; Philadelphia: pp. 1411–1454.

(37) Steer, K.A., Sochor, M. and Mclean,P.,1985.Renal hypertrophy in experimental diabetes :changes in pentose phosphate pathway activity . Diabetes.,34, 485-490.

(38) Paeivansalo, M.J., Merikanto, J., Savolainen, M.J., Lilja, M., Rantala, A.O., Kauma, H., Reumanen, A., Kesaeniemi, Y.A. and Suramo,I.,1998. Effects of hypertension, diabetes and other cardiovascular risk factors on kidney size in middle-aged adults.*Clin.Nephrology*,50 (3),161-168.

(39) Toto,R.D.,1994.Renal insufficiency due to angiotensin converting enzyme inhibitors.*Miner Electrolyte-Metab*.20,193-200.

(40) Kim, H.J.,1994. Mechanisms of hyperkalemia associated with hyporeninemic hypoaldosteronism in streptozotocin diabetic rats. *J.Korean.Med.Sci*., 9(2),107-115.

(41) Jenkins, A.L., Leiter, L.A. and Francis, T., 2001. American ginseng (Panax quinquefolius) attenuates postprand glycemia in a time-dependant manner in healthy individuals . *Am.J.Clin.Nutr*., 73(4),753-758.

(42) Nariman, K.B., 1997. Effect of panax ginseng on the nephrotoxicity of streptozotocin-induced experimental diabetes. *The Egyptian Journal of Biochemistry*.,15(1,2), 29-52

(43) Jin, L.Q. and Shi, L.1991. Effects of saponins of panax notoginseng on sodium-potassium-adenosine triphosphatase and calcium-magnesium-adenosine triphosphatase. *Chung-Kuo-Yaa-Li-Hsueh-Pao*,12(6),504-506.

(44) Mansour, H. A., and Newairy, A.A.,,2000. Amelioration of impaired renal function associated with diabetes by Balanites aegyptiaca fruits in streptozotocin –induced-diabetic rats.*J.Med.Res.Inst*., 21(4),115-125.

(45) Ku, D. D., Seller, B. M. and Meezan, E., 1986. Development of renal hypertrophy and increased renal Na-K –ATPase in streptozotocin diabetic rats .*Endocrinology*., 119,672-679.

(46) Weeson, L.G., 1989. Compensatory growth and other growth response of the kidney .*Nephron*., 51, 149-184.

(47) Christoph, J.O. and Beate, G., with the technical assistance of Elisabeth, G, 1992. Renal hypertrophy in streptozotocin diabetic rats :Role of proteolytic lysosomal enzymes. *Kidney Int*. 41, 966-972.

(48) Wolf, G. and Ziyadeh, F.N.,1999. Molecular mechanisms of diabetic renal hypertrophy. *Kidney Intr –Supplement* .,56(2),393-405.

(49) Subbaih, P., Ramji. L.K., and Joseph,F.A.,1991.Insulin-like effect of vanodate on malic enzyme and glucose-6-phosphate dehydrogenase activities in strepotozotocin –induced diabetic rats liver.*Biochem.Biophy.Acta*.,1083,310-312.

(50) Hikino, H., Ishiyama, M., Suzuki, Y. and Konno,C.,1989.Antidiabetes drugs .Part 33.Validity of oriental medicines. Part 135.Mechanism of hypoglycemic activity of ganoderan B: a glycan of Ganoderma lueidum fruit bodies. *Planta Med*., 55 (5),423-428.

(51) Shibib, A.P., Khan, A.L. and Rahman, R., 1993. Hyperglycemic activity of Coccinia indica and Momordica charanita in diabetic rats;depression of the hepatic gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase and elevation of both liver and red cell shunt enzyme glucose-6-phosphate dehydrogenase.*Boochem.J.*, 292, 267-270.

(52) Lee, h., Kwon, S. and Lee,H.,1997.Hypoglycemic action of components from red ginseng : Investigation of the effect of ginsenosides from red ginseng on enzymes related to glucose metabolism in cultured hepatocytes .*Koryo Insam Hakhoechi*., 21(3),174-186.

(53) Chung, S.H., Choi, C.G. and Park,S.H.,2001.Comparisons between white ginseng radix and rootlet for antidiabetic activity and mechanism in KKAy mice. *Arch. Pharm. Res*.24 (3), 214-218.

(54) Ludvigson, M.A., and Sorenson, R.L., 1980.Immunohiostochemical localization of aldose reductase. *Diabetes*. 29,450-459.

(55) Lim, S.S., Jung, S.H., Ji,J., Shin, K.H., Keum, S.R., 2001. Synthesis of flavonoids and their effect on aldose reductase and sorbitol accumulation in streptozotocin-induced –diabetic rat tissues. *Journal of pharmacy and pharmacology*. 53(5),653-668.

(56) Nishimura-Yabe, 1998.Aldose reductase in the polyol pathway; a potential target for the therapeutic intervention of diabetic complications. Nihon yakurigaku zasshi. 111,137-145.

(57) Hodgki, A.D., Sondergaard, K.L., Yang, B.M., Crass, D.F., Millward, B.A., Demaine, A.G., 2001.Aldose reductase expression is induced by hyperglycemia in diabetic nephropathy. Kidney International, 60(1), 211-218.

(58) Wallner, E.A., Wada, J., Tramonti, G., Lin, S., Srivastava, S.K., Kanwar, Y.S., 2001. RElevance of aldoketo reductase family members to the pathobiology of diabetic nephropathy and renal development of renal failure. Renal Failure, 23(3,4), 311-320.

(59) Meyer, C., Woerle, H.J., Dastou, J.M., Welle, S.L., and Gerich, J.E., (2004) Abnormal renal, hepatic, and muscle glucose metabolism following glucose ingestion in type 2 diabetes. *Am J. Phyiol Endocrinol Metab*, 287 (6), 1049-56.

(60) Han, H.J., Park, S.H., Koh, H.J., Nah, S.Y., Shin, D.H., and Choi H.S., 1999. Protopanaxatrial ginsenosides inhibits glucose uptake in primary cultured rabbit renal proximal tubular cells by arachidonic acid release. *Kidney Blood Press.Res*., 22 (3),114-120.

(61) Hou, J.P., 1977. the chemical constituents of ginseng plants. *Med. East West*.,5, 123-145.

(62) Yuan, H.D., Chung, S.H., 2012. Fermented ginseng protects streptozotocin-induced damage in rat pancreas by inhibiting NF-_B. *Phytomedicine* (19) 494– 505.

Table 1: Changes in the serum levels of glucose, K^+ and Na^+ and kidney weight of rats in different experimental groups.

Assayed Parameters	Experimental groups						
	Control (C)	Diabetic 7 days (1)	GRE treated 7 days (4)	Diabetic 14 days (2)	GRE treated 14 days (5)	Diabetic 21 days (3)	GRE treated 21 days (6)
glucose (mg/dl)	84.4±9.8	$346.3±20.6^{a}$*	$272.7±15.8^{b}$**	$378±15.6^{a}$*	$93.02±16.7^{b}$*	$378.8±13.3^{a}$*	$86.9±4.9^{b}$*
K^+ (mg/dl)	17.1±0.86	$21.4±0.76^{a}$*	$19.81±0.5^{b}$***	$21.1±0.76^{a}$*	$18.17±0.8^{b}$**	$19.23±0.9^{a}$*	$15.68±1.07^{b}$**
Na^+ (mg/dl)	336.7±10	$295.3±7.56^{a}$*	$313.7±3.15^{b}$**	$312.7±3.5^{a}$*	$326±3.7^{b}$**	$318.14±5.6^{a}$*	$333.4±6^{b}$***
Kidney wt. (gm)	0.82±0.08	$1.18±0.12^{a}$*	$0.92±0.04^{b}$**	0.98±0.06	0.9±0.12	0.98±0.1	0.96±0.02

Values are means ± S.D.

A: The means are significantly different from control

B: The means are significantly different from diabetic group

*: P<0.0001, **: P<0.001, ***:P<0.01

P>0.05 is non-significant difference.

Table 2: Changes in the activities of G6PDH and 6PGDH in kidney and liver of different experimental groups.

Parameter	Experimental groups						
	Control (gp C)	Diabetic 7 days (gp 1)	GRE treated 7 days (gp 4)	Diabetic 14 days (gp 2)	GRE treated 14 days (gp 5)	Diabetic 21 days (gp 3)	GRE treated 21 days (gp 6)
G.6.PDH in liver (U/g tissue)	3.12±0.23	$2.41±0.12^{a}$**	$2.81±0.09^{b}$*	2.13±0.24a*	$2.39±0.39^{b}$**	$2.04±0.13^{a}$*	$2.93±0.18^{b}$*
6 PGDH in liver (U/g tissue)	5.16±0.46	$3.67±0.11^{a}$*	$4.26±0.27^{b}$*	3.49±0.36a*	$4.58±0.45^{b}$***	$3.33±0.10^{a}$*	$5.05±0.30^{b}$*

6PGDH in kidney (U/g tissue)	0.95±0.06	1.92±0.076[a]**	1.62±0.064[b]***	1.65±0.082a**	1.19±0.054[b]**	0.99±0.061	1.17±0.066[b]***
G6PDH in kidney (U/g tissue)	0.79±0.03	1.59±0.05[a]***	1.39±0.05[b]****	1.6±0.02a**	1.11±0.038[b]**	1.30±0.027[a]*	0.8±0.038[b]**

Values are means ± S.D.

A: The means are significantly different from control

B: The means are significantly different from diabetic group

*: P<0.0001, **: P<0.001, ***:P<0.01

P>0.05 is non-significant difference.

Table 3: Changes in the levels of sorbitol, fructose, glucose and G6P in kidney tissue of different experimental groups.

Parameter	**Experimental groups**						
	Control (gp C)	**Diabetic 7 days (gp 1)**	**GRE treated 7 days (gp 4)**	**Diabetic 14 days (gp 2)**	**GRE treated 14 days (gp 5)**	**Diabetic 21 days (gp 3)**	**GRE treated 21 days (gp 6)**
Sorbitol (µg/g)	45.95±3.62	59.08±1.09[a]**	54.23±1.89[b]****	66.17±1.19[a]**	53.42±1.51[b]**	69.52±2.16[a]**	45.93±2.68[b]**
Fructose (µg/g)	0.31±0.02	0.46±0.004[a]**	0.43±0.002[b]**	0.50±0.008[a]**	0.36±0.004[b]**	0.51±0.009[a]**	0.29±0.009[b]**
Glucose in tissue (mg/g)	0.74±0.13	4.34±0.33[a]**	1.9±0.3[b]***	3.73±0.48[a]**	2.32±0.13[b]****	4.14±0.43[a]**	1.89±0.4[b]**
G.6.P (µg/g)	0.01±0.002	0.025± 0.0013[a]**	0.018± 0.0034	0.029± 0.0029[a]**	0.017± 0.0016[b]**	0.035± 0.0015[a]**	0.012± 0.0021[a]**

Values are means ± S.D.

a: The means are significantly different from control

b: The means are significantly different from diabetic group

*: P<0.0001, **: P<0.001, ***:P<0.01

P>0.05 is non-significant difference.