Ghiravani et al., Afr J Tradit Complement Altern Med. (2016) 13(2):94-100
http://dx.doi.org/10.4314/ajtcam.v13i2.12

EVALUATION OF HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF INTERNAL SEPTUM OF WALNUT FRUIT IN ALLOXAN-INDUCED DIABETIC RATS

[1]Zahra Ghiravani, [2] Mehran Hosseini, [3]Mohamad Mehdi Hassanzadeh Taheri, [4] Mohammad Hassanpour Fard, [5,]*Mohammad Reza Abedini

[1]Department of physiology, school of Medicine, Birjand University of Medical Sciences (BUMS), Birjand, Iran, [2]Department of public health, Research Centre of Experimental Medicine, BUMS, Birjand, Iran, [3]Department of Anatomy, BUMS, Birjand, Iran.
[4] Berberis and Jujube Research Center, Department of Pharmacology, School of Medicine, BUMS, Birjand, Iran,
[5]Cellular and Molecular Research Center, Department of Pharmacology, School of Medicine, BUMS, Birjand, Iran.
*Correspondence to: Mohammad Reza Abedini, Mohammad Hassanpour Fard,

Corresponding Authors E-mails: mohrabe@yahoo.com, mhassanpour61@yahoo.com

Abstract

Background: Todays, the popularity of herbal medicine is increasing worldwide. Due to importance the evaluation of medicinal herbs efficacy and safety, the present study was conducted to investigate the antidiabetic and hypolipidemic effects of internal septum of walnut fruit (ISWF) ethanolic extract in diabetic rats.
Materials and methods: Alloxan diabetic rats treated orally with ethanolic extract of ISWF (0-400mg/kg) for 28 days. To evaluate its anti-diabetic activity, the animals fasting blood glucose were determined on the first, 14th and 29th days. Moreover, oral glucose tolerance test (OGTT) was performed in diabetic rats at the last day of the study. After 24h of last administration, the blood samples were collected, and the Plasma lipids and liver enzymes levels were measured in fasting overnight rats.
Results: The extract significantly decreased blood glucose ($p<0.001$) on 14th and 29th days. In addition, OGTT revealed that the hypoglycemic effect of the extract appeared at 90 minutes. Alanine aminotransferase and aspartate aminotransferase levels significantly decreased in diabetic rats treated with the both doses ($p<0.05$). Both doses of the extract were able to decrease triglyceride significantly ($p<0.05$) in treated diabetic rats, while only the lower dose of the extract (200 mg/kg) attenuate the total cholesterol and low density lipoprotein ($p<0.001$).
Conclusion: These finding support the notion that ISWF is able to reduce hyperglycemia and hyperlipidemia risk in diabetic rats.

Key words: *Juglans regia*, internal septum, walnut fruit, Antidiabetic, Lipid profile

Introduction

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia due to the impaired secretion and/or action of insulin (Chikhi et al., 2014). Its prevalence is increasing in many populations all over the world. In 2011, there were 366 million cases with diabetes, and it is expected to increase up to 522 million by 2030 (Whiting et al., 2011). Hyperglycemia leads to alteration in metabolism of carbohydrate, protein and fat (Wan et al., 2013). This condition results in long term pathogenic disorders such as neuropathy, retinopathy and nephropathy and consequently lowering in life quality (Santaguida et al., 2005). Currently, multiple pharmacological interventions including sulphonylureas, biguanides, alpha-glucosidase inhibitors, thiazolidinediones and/or insulin are applied for the treatment of diabetes (Grossman et al., 2013). However, all of these medications have limited efficacy and result in various side effects such as gastrointestinal upset, weight changes, hypoglycemia, joint stiffness, kidney and skin complications (Nathan, 2007; Soccio et al., 2014).

Complementary and alternative medicine has been often used for treatment of diabetes. The most widely used therapies in diabetic populations are nutritional supplements and herbal; people with diabetes use these therapies concurrently with conventional health care services (Chang et al., 2007).

The fruit of Juglans regia L (family; Juglandaceae) is a valuable nut largely consumed around the world. Not only dry fruits (nuts) but also its green outer pericarp (husks), inner pericarp (shells), barks and leaves have been used in cosmetic and pharmaceutical industries (Stampar et al., 2006). The seeds of Juglans regia L. are highly nutritious which have been widely used in traditional medicine for treatment of venous insufficiency, hemorrhoid, and diarrhea (Petlevski et al., 2001; Sarahroodi, 2012). In Iran, the internal septum of walnut fruit (ISWF) has been traditionally used to treat diabetic patients (Mirheidari, 1995; Zargari, 1990). However, the impact of Junglans regia septum in diabetes has not been fully elucidated. The present study was conducted to evaluate the hypoglycemic and hypolipidemic effects of internal septum of Junglans regia in alloxan-induced diabetic rats.

http://dx.doi.org/10.4314/ajtcam.v13i2.12

Materials and Methods

Plant Collection and Extract Preparation

The internal septum of Walnut fruit (ISWF) was purchased from traditional medicine shopkeeper in local market of South Khorasan province, Birjand, Iran and confirmed by an expert botanist, and a voucher specimen (721) was assigned by the herbarium of Agricultural school in Birjand University, Birjand, Iran. The ISWF was dried in shade, powdered by electric grinder and then the powder was macerated (1:10 w/v) in ethanol 80% at room temperature with occasionally shaking for 2 days. Afterwards, the mixture was filtered (Blue Ribbon,Grade 589;Germany), from which 10 ml of the concentrated extract was transferred and dried in a Petri dish at 40 °C temperature. The yield of dried extract was about 5% (w/w).

Total Phenol Assay of the Extract

Phenolic compounds in the ethanolic extract of ISWF were assessed by Folin-Ciocalteu's method (Živković *et al.*, 2009). Results were expressed as Means ± Standard Deviation (S.D.) of three parallel replicates for total polyphenol contents. Gallic acid was used as a standard and the total phenolics content was expressed as mg/g gallic acid equivalents (GAE).

Chemicals

Alloxan and metformin tablets were purchased from Sigma Aldrich (USA) and Merck Sante' s.a.s., (France), respectively. Alloxan and metformin were freshly dissolved in 0.9% normal saline solution for intra-peritoneal (ip) and oral administration respectively.

Animals and Experimental Protocol

Male albino Wistar rats with body weight 180-220 g were used in this study. Animals were housed in poly ethylene cages at temperature 21-25^{0}C, 12 h light/dark cycle and relative air humidity 40-45%. Rats fed at libido with the standard food and tap water. The experimental procedure used in the present study approved by the ethic committee of laboratory animal research at Birjand University of Medical Sciences**.**

Induction of Experimental Diabetes

Diabetes was induced in overnight fasted rats using freshly prepared alloxan monohydrate (ip) dissolved in 0.9% saline at a single dose (150 mg/kg). After 14 days of injection, the rats with fasting blood sugar (FBS) more than 350 mg/dl were allocated as severe diabetic (Etuk, 2010). Similarly, the control group consisting of 8 rats was injected by normal saline 0.9%.

Experimental Designs

Assessment of ISWF on Alloxan Induced-Diabetic Rats

Thirty two diabetic and 8 normal animals were randomly divided into 5 equal treatment as well as control groups respectively. Then the animal groups received treatment as follows: 1: healthy rats (saline 0.9%); 2: diabetic rats (saline 0.9%); 3: diabetic rats treated with metformin at 50 mg/kg body weight (b.wt) as positive control; 4 and 5: diabetic rats treated with ethanolic extract of ISWF with 200 and 400 mg/kg b.wt respectively.

The ethanolic extract of ISWF was daily administered for 4 weeks by oral gavage. The blood samples for FBS measurement were collected from tail vein at the first (before treatment), 14th and 29th after treatment. The glucose levels were determined using ACCU CHEK Active gluco-meter (Germany).

Study on Oral Glucose Tolerance Test (OGTT)

For OGTT assessment, 4 equal groups (n=8) were chosen and orally treated for 28 days (daily) as follows: 1) healthy rats with saline 0.9%. as normal control, 2) diabetic rats with 50 mg/kg metformin as positive control, 3 and 4) diabetic rats with 200 mg and 400 mg/kg of ISWF as intervention. OGTT was performed in overnight fasted (18 h) rats. After 30 min of treatment, all the groups were orally loaded with 3 g/kg of glucose. Blood samples were collected 0-120 minutes after glucose loading. Blood glucose levels were measured using ACCU CHEK Active glucose oxidase-peroxidase reactive strips (Germany).

Effects of ISWF on Lipid Profile and Liver Enzymes

On 29th day, after 24h of last administration, fasted overnight rats were anesthetized and blood samples were drawn from heart. Lipid profile; total cholesterol, triglyceride (TG), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and liver enzymes; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed using Pars Azmoon standard kits (Iran) and Liasys Random Access AMS auto-analyzer (Italy).

Statistical Analysis

All the data were expressed as mean ± S.D. Data were analyzed by SPSS software (version 16) using ANOVA and tukey post hoc test and the difference were considered significant at $p<0.05$.

http://dx.doi.org/10.4314/ajtcam.v13i2.12

Results

Total Polyphenol Contents

Although internal septum of walnut fruit (ISWF) has been traditionally used to treat diabetes, its influence in metabolic disorders has not been fully investigated. To examine the effect of ISWF on metabolic disorders, we first measured the total polyphenol content of its ethanolic extract and showed that each gram of dried extract contained 21.64 ± 1.44mg GAE/g.

Hypoglycemic Effects of ISWF on Alloxan Induced-Diabetic Rats

We next analyzed the effect of ISWF on FBS concentration in alloxan-induced diabetic animals. As it is shown in Fig. 1, on the first day before any treatment, there was a significant increase as much as 4-5 times on FBS levels in experimental groups which received alloxan in comparison with control (healthy) group. After 14 days of alloxan administration, ISWF extract significantly decreased blood glucose levels in a dose-dependent manner in comparison to diabetic control rats ($p<0.05$). Metformin (50 mg/kg, as a positive control) also significantly decreased FBS level ($p<0.05$). On the 29th day, both doses of the extract and metformin were able to decrease FBS level significantly in diabetic rats when compared with diabetic control group ($p<0.05$). This response in the extract groups were more than metformin group on 14th and 29th days; however, these differences were not significant ($p>0.05$).

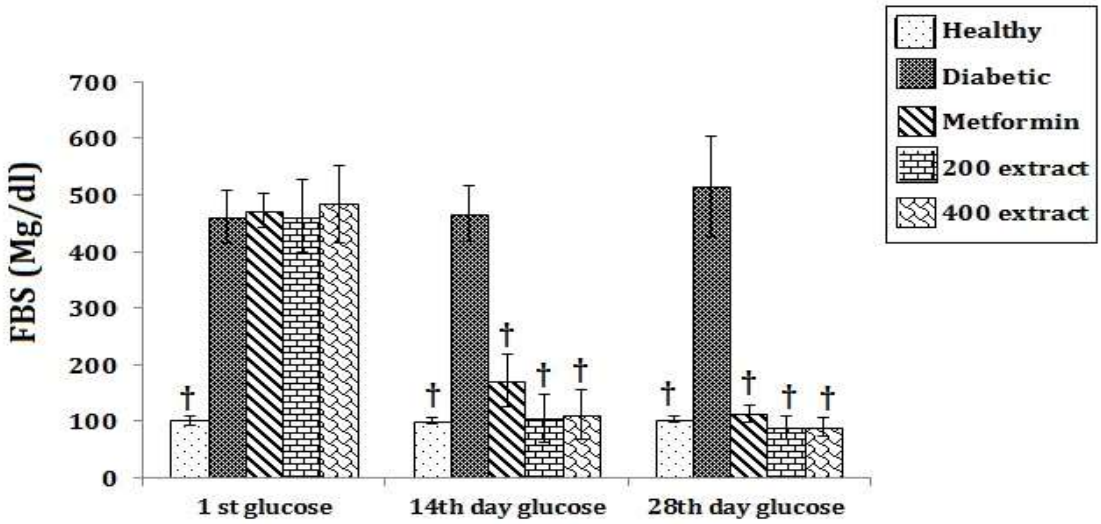

Figure 1

Figure 1: Internal septum of walnut fruit (ISWF) exerts anti-diabetic activity in alloxan-induced severe diabetic rats. Animals were treated with normal saline 0.9% as a vehicle (Healthy and diabetic control groups), metformin (50 mg/kg body weight, as positive control) and ethanolic extract of ISWF (200 and 400 mg/kg body weight). FBS levels were measured on the 1st, 14th and 29th days using a glucometer. Each column represents mean ± S.D, for 8 rats.† $P<0.001$ compared with diabetic control group.

Effect of Ethanolic Extract of ISWF in OGTT in Normal Rats

To examine hypoglycemic efficacy of ISWF, OGTT was performed in diabetic rats. Table 1 shows the blood glucose levels (BGL) of the control, Metformin (50mg/kg) and ethanolic extracts of ISWF (200 mg and 400mg/kg) at different time points (0, - 120 min). While oral glucose loading (3g/kg) significantly increased BGL in diabetic rats with a peak at 30 min, ISWF dramatically restored glucose levels to normal status in 90 and 120min at concentrations of 400 and 200mg/kg respectively. These responses were dose-dependent and comparable with metformin.

Table 1: Effect of ISWF in oral glucose tolerance in diabetic rats

S.N0	Group (treatment)	Blood glucose level mg/dl (Mean ± SD)				
		fasting	Post treatment			
			30 min	60 min	90 min	120 min
1	Control (saline0.9%)	82.58 ± 3.65	86.66 ± 4.22	84.38 ± 1.25	85.65 ± 1.01	84.63 ± 3.68
2	Metformin (50mg/kg) + glucose	79.46 ± 2.13	* 199.85 ± 4.29	* 155.43 ± 3.62	* 105.54 ± 4.25	81.8 ± 3.81
3	ISWF(200mg/kg) + glucose	81.23 ± 2.81	* 201.67 ± 2.51	* 154.28 ± 3.17	* 100.97 ± 1.99	92.17 ± 1.21
4	ISWF(400mg/kg) + glucose	79.69 ± 3.89	* 193.23 ± 1.93	* 143.78 ± 2.32	93.21 ± 2.44	78.21 ± 0.89

Values are expressed as mean ± SD. ANOVA followed by Tukey test. * $P \leq 0.05$ compared normal control group

http://dx.doi.org/10.4314/ajtcam.v13i2.12

Effects of ISWF on Lipid Profile and Liver Enzymes

To examine the effect of ISWF on the liver enzyme activity, the above experiment extended to assess the effect of ISWF extract on plasma AST and ALT levels in diabetic rats. As shown in Fig. 2, the plasma levels of AST and ALT increased significantly in diabetic rats when compared with healthy group after 29 days. Administration of 200 and 400 mg/kg body weight of ISWF ethanolic extract dramatically decreased AST in a dose-dependent manner ($p=0.013$ and $p=0.025$, respectively) and ALT ($p<0.001$) in comparison to diabetic control group. Although administration of metformin at the dose of 50mg/kg body weight did not alter AST ($p=0.058$), it significantly reduced ALT in comparison to diabetic group ($p<0.001$).

As it is shown in Figure 3, the plasma total cholesterol, triglyceride and LDL-c significantly increased in diabetic rats in comparison to healthy control rats. The ISWF extract significantly decreased total cholesterol ($p<0.001$), TG ($p=0.001$) and LDL-c ($p<0.001$) at the dose of 200 mg/kg compared with diabetic rats. At the dose of 400 mg/kg, the extract only dramatically decreased TG level ($p=0.017$), while metformin did not significantly alter any of the elevated parameters.

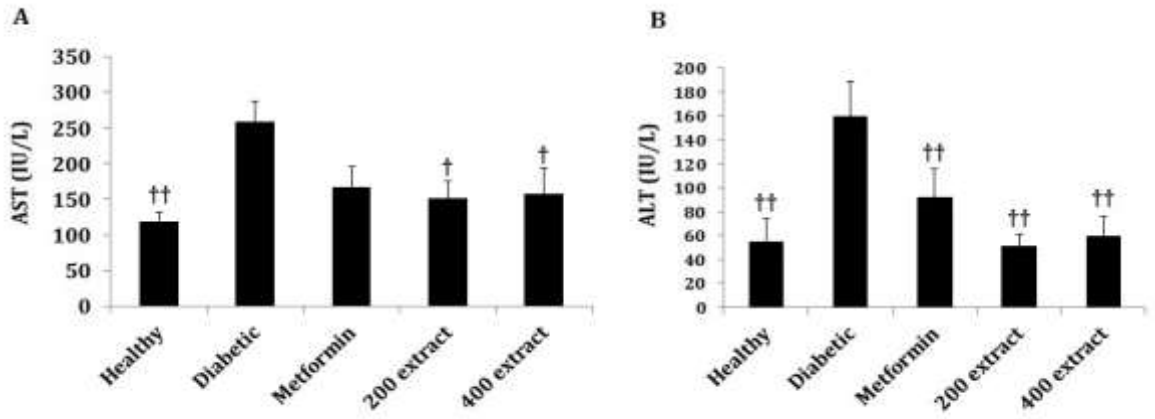

Figure 2

Figure 2: ISWF decreases plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in diabetic rats. Animals were treated with normal saline 0.9% as a vehicle (Healthy and diabetic control groups), metformin (50 mg/kg body weight, as positive control) and ethanolic extract of ISWF (200 and 400 mg/kg body weight). AST (Fig, 2A) and ALT (Fig, 2B) levels were assessed at 29th day. Each column represents mean ± S.D, for 8 rats. † $p<0.05$ and †† $p<0.001$compared with diabetic control group.

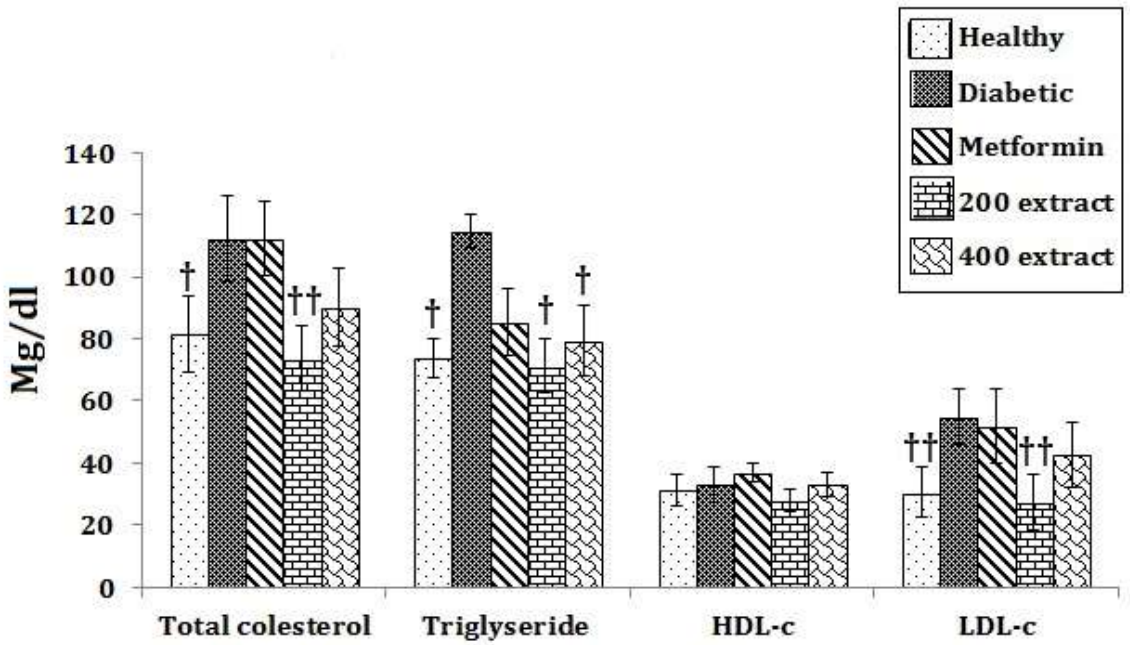

Figure 3

Figure 3: Oral administration of ethanolic extract of ISWF attenuates plasma lipid profile in diabetic rats. Animals were treated with ethanolic extract of ISWF (200 and 400 mg/kg body weight), normal saline 0.9% as a vehicle (Healthy and diabetic control groups) and metformin (50 mg/kg body weight, as positive control). Total cholesterol, triglyceride (TG), high density lipoprotein cholesterol (HDL-c) and low density lipoprotein (LDL-c) were analyzed on the 29thdaysin diabetic rats. Each column represents mean ±S.D. for 8 rats. † $p<0.05$ and †† $p<0.001$ different from control group.

http://dx.doi.org/10.4314/ajtcam.v13i2.12

Discussion

We have studied the influence of ISWF extract on metabolic disorders and demonstrated that it significantly decreased the serum levels of ***a)*** blood glucose in a dose-dependent manner, ***b)*** AST and ALT liver enzymes, and ***c***) total cholesterol, TG and LDL-c in alloxan-induced diabetes when compared with diabetic control rats.

Diabetes can be induced by pharmacological, surgical or genetic manipulation in several animal species. The most experiments on diabetes were carried out in rodents (Eddouks *et al.*, 2012; Shirwaikar *et al.*, 2006). The majority of studies have applied pharmacological models in which streptozotocin or alloxan most frequently have been used for diabetes induction (Shirwaikar *et al.*, 2006). Both drugs exert their diabetogenic action through reactive oxygen species, which cause rapid destruction of pancreatic β-cells(Szkudelski, 2001). In the present study, alloxan has been used to induce diabetes in animal as reported previously (Etuk, 2010).

The ethanolic extract of ISWF significantly decreased glucose level in treated diabetic rats as compared with control group (Fig. 1). Anti-hyperglycemic activity of ISWF may be due to presence of polyphenolic compounds in high level (21.65 ± 1.44mg GAE/g). The other studies shown that several antidiabetic plants have high levels of polyphenols (El-Demerdash *et al.*, 2005; Jelodar *et al.*, 2005) such as; garlic (17.38mg/g), onion (22.32 mg/g) and fenugreek (198 mg/g) (Cheng *et al.*, 2013; Lu *et al.*, 2011; SEASOTIYA *et al.*, 2014). Polyphenol has also improved insulin–dependent glucose uptake in muscle cells and adipocytes by translocation of glucose transporter, GLUT4, to plasma membrane mainly through induction of the AMP-activated protein kinase (AMPK) pathway(Park *et al.*, 2007; Zhang *et al.*, 2011). AMPK, an important sensor of cellular energy status, has a key role in metabolic control through the activation of this pathway and is considered as a new treatment for obesity, diabetes, metabolic syndrome and main target for anti-diabetic drugs including metformin (Kumar *et al.*, 2009; Zang *et al.*, 2006). Polyphenols also inhibit α-glucosidase and α-amylase, the enzymes responsible for digestion of dietary carbohydrate to glucose (Tadera *et al.*, 2006). Plant-food polyphenols have been shown to attenuate hepatic gluconeogenesis via decreasing the activity of glucose-6-phosphatase and phosphoendolpyruvate carboxykinase (PEPCK) and down regulation of liver glucokinase expression (Waltner-Law *et al.*, 2002). Several studies have shown that polyphenol compounds protect β-cells from oxidative-induced damages by enhancing the natural antioxidant system, inhibition of lipid peroxidation and stimulation on the β-cells (Szkudelski, 2007; Szkudelski and Szkudelska, 2011). These suggest that the possible mechanisms for antidiabetic action of ISWF may be due to stimulation of peripheral glucose uptake in tissues, modulation of carbohydrate metabolism and attenuation of intestinal absorption for dietary carbohydrate. Also it may act by increasing the insulin secretion from the existing pancreatic β cells. To our knowledge, this is the first report that shows ISWF completely reduce elevated glucose serum level in diabetic rats to normal value.

Our finding also showed that ISWF significantly prevented the elevated AST and ALT serum levels in treated diabetic rats as compared with control group. These enzymes are usually found in large quantities in the liver where they play an important role in the metabolism of amino acids (Yin *et al.*, 2011). Due to damage or toxicity to the liver (*e.g.* in diabetic patients), these enzymes may leak from the hepatocytes into the circulation, and lead to elevation in blood (Carobene *et al.*, 2013; Coballase-Urrutia *et al.*, 2011). In our study similar to the several other studies with different duration (2-8 weeks) and various diabetogenic drugs (alloxan or streptozotocin), AST and ALT significantly elevated in diabetic rats in comparison with control ones(Aloulou *et al.*, 2012; Erejuwa *et al.*, 2012; Karthik and Ravikumar, 2011; Saeed *et al.*, 2008). Interestingly, ISWF not only prevented the elevation of enzyme levels in diabetic rats but also restored their levels to normal values (Fig. 2). In the other study, oral administration of Walnut extract significantly reduced serum glutamic oxaloacetic transaminase (AST) and glutamic pyruvic transaminase (ALT) in injured liver induced by carbon tetrachloride (CCl_4) in mice(Shimoda *et al.*, 2009).

Since lipid abnormalities are the major causes of cardiovascular diseases in diabetic patients, ideal treatment for diabetes (in addition to glycemic control) should have a favorable effect on lipid profile. The various types of oral anti-diabetic drugs, currently approved for the treatment of type 2 diabetes do not have a desire effects on cardiovascular disease(Fisman *et al.*, 2008). while most of anti-hypercholesterolemic drugs do not decrease TG levels (El-Hazmi and Warsy, 2001), the ethanolic extract of ISWF in both doses prevented the elevation of plasma TG level after 28 days of administration (Fig. 3). In terms of total cholesterol and LDL-c levels, IWSF at the dose of 200 mg/kg significantly reduced these values after 28 days of treatment. Other studies also shown that walnut diet reduced the levels of, LDLc, and HDLc, total cholesterol as well as the ratio of LDL cholesterol to HDL cholesterol(Sabate *et al.*, 1993; Tapsell *et al.*, 2004). Walnut also has been reported to have several phytosterol compounds which are able to inhibit intestinal absorption of cholesterol (Amaral *et al.*, 2003). Oral administration of walnut polyphenol significantly reduced liver and serum triglycerides (Shimoda *et al.*, 2009). The lipid lowering effect of the extract might be due to the action of flavanoids and other phenolic compounds. The plant polyphenols reduce cholesterol level by inhibiting 3-hydroxy-3-methyl-glutaryl Coenzyme A reductase (HMG CoA reductase), the rate-regulatory enzyme of cholesterol biosynthesis (Sharma *et al.*, 2003). Moreover, it was shown that some polyphenols such as tri-terpenoids can decrease TG levels by activated lipoprotein lipase enzymes (Kamanyi *et al.*, 1994).

In summary, the ethanolic extract of ISWF exhibits strong hypoglycemic and hypolipidemic activity in diabetic rats. The ISWF extract potentially can be considered as a hypoglycemic agent. It also alleviates the dyslipidemia parameters, thereby preventing the cardiovascular complications which are common in diabetic patients associated with diabetes**.**

Acknowledgements

This study was supported by a grant from the Research Vice Presidency of Birjand University of Medical Sciences. The authors are thankful to Mr Pouyan, Birjand University, for his assistance to identifying the plant used in the research.

References

1. Aloulou, A., Hamden, K., Elloumi, D., Ali, M. B., Hargafi, K., Jaouadi, B., Ayadi, F., Elfeki, A. & Ammar, E. (2012). Hypoglycemic and antilipidemic properties of kombucha tea in alloxan-induced diabetic rats. BMC Complement Altern Med 12: 63.

2. Amaral, J .S., Casal, S., Pereira, J. A., Seabra, R. M. & Oliveira, B. P. (2003). Determination of sterol and fatty acid compositions, oxidative stability, and nutritional value of six walnut (*Juglans regia* L.) cultivars grown in Portugal. J Agric Food Chem 51(26 :(.7702-7698
3. Carobene, A., Braga, F., Roraas, T., Sandberg, S. & Bartlett, W. A. (2013). A systematic review of data on biological variation for alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transferase. Clin Chem Lab Med 51(10): 1.2007-997
4. Chang, H. Y., Wallis, M. & Tiralongo, E. (2007). Use of complementary and alternative medicine among people living with diabetes: literature review. J Adv Nurs 58(4): 307-319.
5. Cheng, A., Chen, X., Jin, Q., Wang, W., Shi, J. & Liu, Y. (2013). Comparison of Phenolic Content and Antioxidant Capacity of Red and Yellow Onions. Czech J. Food Sci. Vol 31(5): 501-508.
6. Chikhi, I., Allali, H., El Amine Dib, M., Medjdoub, H. & Tabti, B. (2014). Antidiabetic activity of aqueous leaf extract of *Atriplex halimus* L. (Chenopodiaceae) in streptozotocin–induced diabetic rats. Asian Pacific Journal of Tropical Disease 4(3): 181-184.
7. Coballase-Urrutia, E., Pedraza-Chaverri, J., Cardenas-Rodriguez, N., Huerta-Gertrudis, B., Garcia-Cruz, M. E., Ramirez-Morales, A., Sanchez-Gonzalez, D. J., Martinez-Martinez, C. M., Camacho-Carranza, R. & Espinosa-Aguirre, J. J. (2011). Hepatoprotective effect of acetonic and methanolic extracts of Heterotheca inuloides against CCl(4)-induced toxicity in rats. Exp Toxicol Pathol 63(4.370-363 :(
8. Eddouks, M., Chattopadhyay, D. & Zeggwagh, N. A. (2012). Animal Models as Tools to Investigate Antidiabetic and Anti-Inflammatory Plants. Evidence-Based Complementary and Alternative Medicine 2012: 14.
9. El-Demerdash, F. M., Yousef, M. I. & El-Naga, N. I. (2005). Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem Toxicol 43(1): 57-63.
10. El-Hazmi, M. A. & Warsy, A. S. (2001). Evaluation of serum cholesterol and triglyceride levels in 1-6-year-old Saudi children. J Trop Pediatr 47(3): 181-185.
11. Erejuwa, O., Sulaiman, S., Wahab, M., Sirajudeen, K., Salleh, M. & Gurtu, S. (2012). Hepatoprotective effect of tualang honey supplementation in streptozotocin-induced diabetic rats. 2012 4(4): 5.
12. Etuk ,E. (2010). Animals models for studying diabetes mellitus. Agric Biol JN Am 1(2): 130-134.
13. Fisman, E. Z., Motro, M. & Tenenbaum, A. (2008). Non-insulin antidiabetic therapy in cardiac patients: current problems and future prospects. Adv Cardiol 45: 154-17.0
14. Grossman, A., Johannsson, G., Quinkler, M. & Zelissen, P. (2013). Therapy of endocrine disease: Perspectives on the management of adrenal insufficiency: clinical insights from across Europe. Eur J Endocrinol 169(6): R165-175.
15. Jelodar, G. A., Maleki, M ,.Motadayen, M. H. & Sirus, S. (2005). Effect of fenugreek, onion and garlic on blood glucose and histopathology of pancreas of alloxan-induced diabetic rats. Indian J Med Sci 59(2): 64-69.
16. Kamanyi, A., Njamen, D. & Nkeh, B. (1994). Hypoglycaemic properties of the aqueous root extract of *Morinda lucida* (Benth) (Rubiaceae). Studies in the mouse. Phytotherapy Research 8(6): 369-371.
17. Karthik, D. & Ravikumar, S. (2011). A study on the protective effect of Cynodon dactylon leaves extract in diabetic rats. Biomed Environ Sci 24(2): 190-199.
18. Kumar, R., Balaji, S., Uma, T. S. & Sehgal, P. K. (2009). Fruit extracts of Momordica charantia potentiate glucose uptake and up-regulate Glut-4, PPAR gamma and PI3K. J Ethnopharmacol 126(3): 533-537.
19. Lu, X., Ross, C. F., Powers, J. R., Aston, D. E. & Rasco, B. A. (2011). Determination of total phenolic content and antioxidant activity of garlic (*Allium sativum*) and elephant garlic (*Allium ampeloprasum*) by attenuated total reflectance-Fourier transformed infrared spectroscopy. J Agric Food Chem 59(10): 5215-5221.
20. Mirheidari, H. (1995). Knowledge of Plants. Tehran: Amir Kabir Press.
21. Nathan, D. M. (2007). Finding new treatments for diabetes--how many, how fast... how good? N Engl J Med 356(5): 437-440.
22. Park, C. E., Kim, M. J ,.Lee, J. H., Min, B. I., Bae, H., Choe, W., Kim, S. S. & Ha, J. (2007). Resveratrol stimulates glucose transport in C2C12 myotubes by activating AMP-activated protein kinase. Exp Mol Med 39(2): 222-229.
23. Petlevski, R., Hadzija, M., Slijepcevic, M. & Juretic, D. (2001). Effect of 'antidiabetis' herbal preparation on serum glucose and fructosamine in NOD mice. J Ethnopharmacol 75(2-3): 181-184.
24. Sabate, J., Fraser, G. E., Burke, K., Knutsen, S. F., Bennett, H. & Lindsted, K. D. (1993). Effects of walnuts on serum lipid levels and blood pressure in normal men. N Engl J Med 328(9): 603-607.
25. Saeed, M. K., Deng, Y. & Dai, R. (2008). Attenuation of Biochemical Parameters in Streptozotocin-induced Diabetic Rats by Oral Administration of Extracts and Fractions of Cephalotaxus sinensis. Journal of Clinical Biochemistry and Nutrition 42(1): 21-28.
26. Santaguida, P. L., Balion, C., Hunt, D., Morrison, K., Gerstein, H., Raina, P., Booker, L. & Yazdi, H. (2005). Diagnosis, prognosis, and treatment of impaired glucose tolerance and impaired fasting glucose. Evid Rep Technol Assess (Summ) (128): 1-11.
27. Sarahroodi, S. (2012). Water extract of *Juglans regia* L. and diabetes mellitus in Iranian traditional medicine. Qom University of Medical Sciences Journal 3(3.(
28. SEASOTIYA, L., SIWACH, P., BAI, S., MALIK, A., BHARTI, P. & DALAL, S. (2014). Free radical scavenging activity, phenolic contents and phytochemical analysis of seeds of *Trigonella foenum* graecum.
29. Sharma, S. B., Nasir, A., Prabhu, K. M., Murthy, P. S. & Dev, G. (2003). Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of Eugenia jambolana in alloxan-induced diabetic rabbits. J Ethnopharmacol 85(2-3): 201-206.
30. Shimoda, H., Tanaka, J., Kikuchi, M., Fukuda, T., Ito, H., Hatano, T. & Yoshida, T. (2009). Effect of polyphenol-rich extract from walnut on diet-induced hypertriglyceridemia in mice via enhancement of fatty acid oxidation in the liver. J Agric Food Chem 57(5): 1786-1792.
31. Shirwaikar, A., Rajendran, K. & Barik, R. (2006). Effect of aqueous bark extract of *Garuga pinnata* Roxb. in streptozotocin-nicotinamide induced type-II diabetes mellitus. J Ethnopharmacol 107(2): 285-290.
32. Soccio, Raymond E., Chen, Eric R. & Lazar, Mitchell A. (2014). Thiazolidinediones and the Promise of Insulin Sensitization in Type 2 Diabetes. Cell Metabolism 20(4): 573-591.
33. Stampar, F., Solar, A., Hudina, M., Veberic, R. & Colaric, M. (2006). Traditional walnut liqueur–cocktail of phenolics. Food Chemistry 95(4): 627-631.
34. Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 50(6): 537-546.

http://dx.doi.org/10.4314/ajtcam.v13i2.12

35. Szkudelski, T. (2007). Resveratrol-induced inhibition of insulin secretion from rat pancreatic islets: evidence for pivotal role of metabolic disturbances. Am J Physiol Endocrinol Metab 293(4): E901-907.
36. Szkudelski, T. & Szkudelska, K. (2011). Anti-diabetic effects of resveratrol. Annals of the New York Academy of Sciences 1215(1): 34-39.
37. Tadera, K., Minami, Y., Takamatsu, K. & Matsuoka, T. (2006). Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. J Nutr Sci Vitaminol (Tokyo) 52(2): 149-153.
38. Tapsell, L. C., Gillen, L. J., Patch, C. S., Batterham, M., Owen, A., Bare, M. & Kennedy, M. (2004). Including walnuts in a low-fat/modified-fat diet improves HDL cholesterol-to-total cholesterol ratios in patients with type 2 diabetes. Diabetes Care 27(12): 2777-2783.
39. Waltner-Law, M. E., Wang, X. L., Law, B. K., Hall, R. K., Nawano, M. & Granner, D. K. (2002). Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. J Biol Chem 277(38): 34933-34940.
40. Wan, L. S., Chen, C. P., Xiao, Z. Q., Wang, Y. L., Min, Q. X., Yue, Y. & Chen, J. (2013). *In vitro* and *in vivo* anti-diabetic activity of *Swertia kouitchensis* extract. J Ethnopharmacol 147(3): 622.630-
41. Whiting, D. R., Guariguata, L., Weil, C. & Shaw, J. (2011). IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res Clin Pract 94(3): 311-321.
42. Yin, P., Zhao, S., Chen, S., Liu, J., Shi, L., Wang, X., Liu, Y & .Ma, C. (2011). Hypoglycemic and hypolipidemic effects of polyphenols from burs of *Castanea mollissima* Blume. Molecules 16(11): 9764-9774.
43. Zang, M., Xu, S., Maitland-Toolan, K. A., Zuccollo, A., Hou, X., Jiang, B., Wierzbicki, M., Verbeuren, T. J. & Cohen, R. A. (2006). Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. Diabetes 55(8): 2180-2191.
44. Zargari, A. (1990). Medical Plants. Tehran: Tehran University Press.
45. Zhang, B., Kang, M., Xie, Q., Xu, B., Sun, C., Chen, K. & Wu, Y. (2011). Anthocyanins from Chinese bayberry extract protect beta cells from oxidative stress-mediated injury via HO-1 upregulation. J Agric Food Chem 59(2): 537-545.
46. Živković, J., Mujić, I., Nikolić, G., Vidović, S. & Mujić, A. (2009). Extraction and analysis of condensed tannins in *Castanea sativa* mill. Journal of Central European Agriculture 10(3): 283-280.