International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.10, pp 46-54, 2017

The Effect of Walnut Oil, Septum and leaves aqueous extract in Alloxan-Induced Diabetic rats

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Abstract : The aim of the present study was to evaluate the effects of walnut oil, septum and leaves aqueous extract on blood glucose in diabetic Wistar male rats induced by alloxan. Diabetes animals were induced by alloxan (75 mg/kg of body weight). Rats were randomly divided into 5 groups (n=5), including normal control, diabetic control, diabetic-walnut oil, diabetic-walnut septum, diabetic -walnut leaves aqueous extract , and were examined for 2 weeks. Blood glucose level was measured in all groups. Blood glucose significantly increased in experimental groups in comparison with normal control groups ($p<0.05$) after alloxan injection. Blood glucose level significantly decreased in diabetic control groups ($p<0.05$) with walnut oil, septum and leaves aqueous extract. It should be noted that consuming walnut oil more reduce blood glucose than other sections of walnut. Results demonstrate that oil, septum and leaves extract of walnut possesses significant antihyperglicemic properties, thus suggesting its beneficial effect in the treatment of diabetes.
Keywords : Walnut oil, Walnut septum, Walnut leaves, Blood glucose, Diabetes, Rat.

Introduction

Diabetes is the most common endocrine disorder characterized by hyperglycemia and Carbohydrate, fat and protein metabolism disorder [1].It is a medical condition in which the body does not efficiently utilize insulin, the hormone responsible for regulating blood sugar. In Type 1 diabetes, the body does not produce insulin, whereas in Type 2 diabetes, the body does not produce enough insulin or cells become resistant to insulin. This, in turn, leads to chronic high blood sugar levels that increase the risk for stroke, heart disease, kidney failure and obesity [2]. Diabetes mellitus is associated with increased prevalence of microvascular complications. Type 1 diabetes mellitus results from cellular mediated autoimmune destruction of pancreatic β-cells of islets of Langerhans and results in loss of insulin production. Therefore, damage to pancreatic b cells due to the release of tumor necrosis factor-a (TNF-a) and interleukin-1 (IL-1) produced by infiltrating macrophages, lymphocytes and monocytes leads to the development of type 1 diabetes mellitus [3].Prevalence of diabetes mellitus type 2 is increasing globally. It was noted that an increase in intake of plant sources of fats and proteins in the diet would reduce the risk of coronary artery disease and diabetes mellitus type 2. Several studies demonstrated that increased intake of mono- and polyunsaturated fatty acids and lower intake of saturated and trans-fatty acids are associated with a lower risk of diabetes mellitus type 2[4].The oral hypoglycemic agents currently used in clinical practice possess characteristic profiles of serious side effects. Hence, there is a need to search for newer anti-diabetic agents that retain therapeutic efficacy and are devoid of side effects [5-6].Herbal traditional medicines derived mainly from plants have played major role in the management of diabetes mellitus [7].Among the nuts, walnut has a high omega-3 unsaturated fatty acid and antioxidant component content, providing nutritional and functional properties including improvement of the

gastrointestinal system, decrease in blood glucose level, eventually leading to a decrease in the risk of cardiovascular disease[8].Walnuts are also used as a traditional therapy for treating cough, stomach ache and cancer in Asia and Europe [3].

The heart benefits of walnuts include lowering cholesterol, increasing the ratio of high-density lipoprotein cholesterol to total cholesterol, reducing inflammation, and improving arterial function [9-11]. An April 2004 clinical study from the University of Barcelona showed that substituting walnuts for monounsaturated fatty acids in a Mediterranean diet improved, and even restored, endothelial function. According to the researchers, walnuts are the first whole food to show such cardiovascular benefits [11]. Cardio-protective dietary fat intakes are recommended as part of the treatment for type 2 diabetes patients [12]. Walnut consumption improves endothelial function in type 2 diabetic individuals and may therefore help reduce cardiovascular disease risk in this high-risk population[13].A diet rich in walnut would have a positive effect on endothelial function and endothelium-dependent vasodilation in diabetes. These dietary changes consequently would reduce the overall risk for coronary artery disease[14].There are few reports regarding the use of walnut in diabetic patients [15]or walnut leaf aqueous extract on blood glucose and The lipid changes in diabetic animals [16-18].

In this study, the effects of walnut oil, septum and leaf aqueous extract on blood glucose in diabetic subjects will be discussed using an animal model.

Materials and Methods

Extract preparation

Walnuts (Juglansregia) used in this experiment were collected in July, 2014 from gardens in Noghondar, Mashhad, Iran, and identified by specialized botanist in the Department of Botany, University of Mashhad .Septum of walnut shell (300g) was separated and shed dried (at 25 ºC) ground and mixed with water by blender. After 24 h, the mixture was filtered through Wattman filter, evaporated by rotatory evaporator and dried in a desiccator. The extract was obtained with the percolation method. The final yield was 30 g powdered extract. The oil content of walnuts was extracted with hexane (Sigma Aldrich)by the extraction method [3]. For walnut leaves aqueous extract preparation all healthy leaves of walnuts were washed and dried in shadeand grinded to powder after well dried. 50 gram of the leave powder was soaked in 1000 ml water and placed on a shaker (VAC 230 model)for twenty-four hours (24hrs) [19].

Animal treatments

Male adult rats (wistar strain), weighting 150-180 g obtained from University of Medical Sciences, Mashhad, Iran, and were kept in ananimal chamber (24±2°C) under the care of experienced animal technicians.

Diabetes induction

Alloxan monohydrate (Sigma) was dissolved in sterile distilled water. Diabetes was induced in 25 rats by intraperitoneal injection of alloxan (5%) 185 mg/kg [20-21]. Experimental diabetes was induced by administration of alloxan monohydrate (75 mg/kg of body weight) [3]intraperitoneally two weeks before starting the treatment. After a 2-week experimental period, the blood glucose level of the animals was tested for evidence of adiabetic state, following a 12 h fast. The animals that had a blood glucose level equal to or greater than 250 mg/dl were included in the study [3]. The study was reviewed and approved by the Ethics Committee of Mashhad University of Medical Sciences. The rats were randomly divided into five groups (n = 5): normal control (normal rats which received distilled water), diabetic control (normal rats given alloxan monohydrate (75 mg/kg of body weight) intraperitoneally, daily for three days), diabetic-walnut oil (diabetic rats given extract of walnut oil (400 mg/kg), daily for two weeks orally by gavage), diabetic-walnut Septum (diabetic rats given extract of walnut septum (400 mg/kg), daily for two weeks orally by gavage),diabetic- walnut leaves aqueous extract (diabetic rats given extract of walnut leaves (400 mg/kg), daily for two weeks orally by gavage).

Blood sample collection and analysis

To Catching investigation of diabetes in rats, blood samples after 3 days of Alloxan injection were collected from end of tail. To investigate the effects of walnuts oil, leaves extract and Septum on diabetes, blood samples were collected three times: time 0 was before injection of alloxan, time 1 was 7days after injection of alloxan and time 2 was 14 days after injection of alloxan. Serum glucose concentration were determined by the glucose oxidase method using glucose oxide kit (zist chime, Tehran, Iran) [22].

Statistical analysis

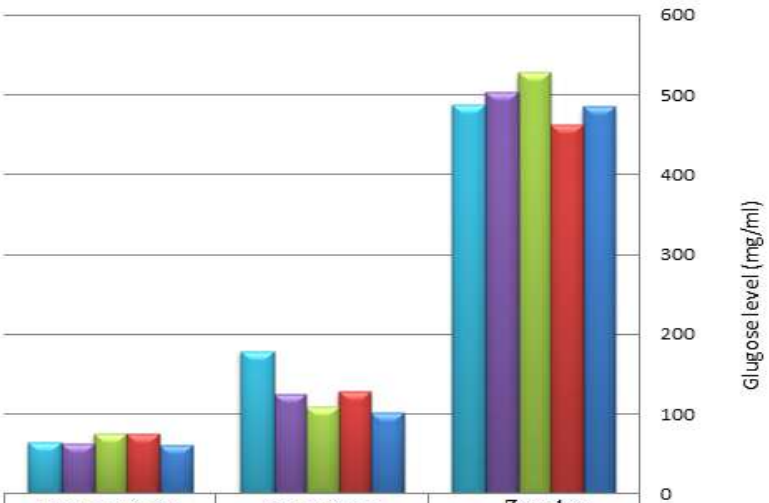
In this study factorial method was employed with the aid of MstatC statistical software to design of experiments. Significant differences among the groups were determined by one way analysis of variance (ANOVA). Values of $P < 0.05$ were taken as statistically significant.

Results and discussion

The results of blood glucose measurement after 3 days of alloxan monohydrate injection into groups of 2 to 5 showed that a part from control group (Group 1),all samples groups of 2 to 5, are suffering from diabetes.

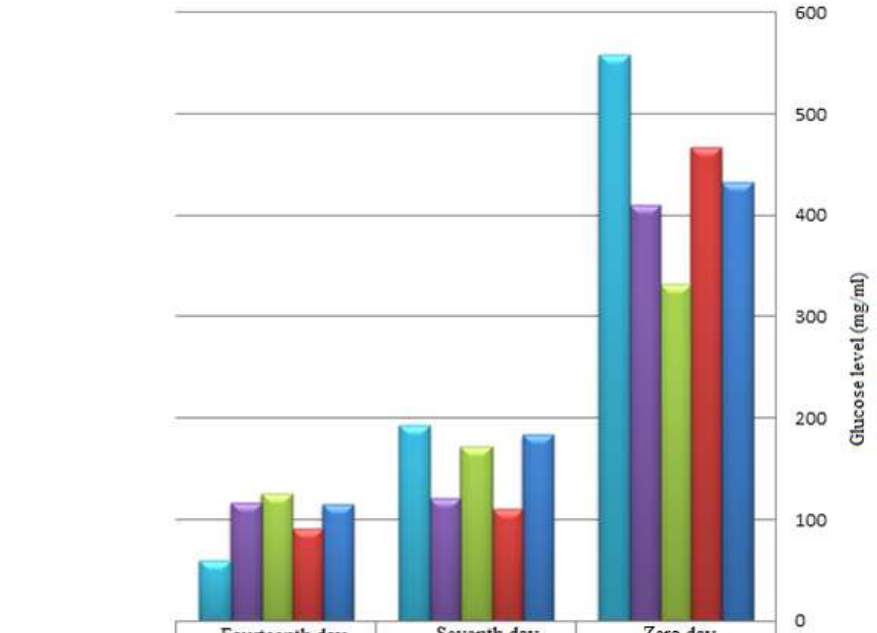
Effects of walnut oil, walnut septum and walnut leaves aqueous extract on serum glucose level in diabetic rats during 14 days of oral administration are shown in Figs 1-3.

As can be seen blood glucose level was found to be significantly decreased in diabetic rats treated with walnut oil, walnut septum and walnut leaves aqueous extract respectively in 14 days.



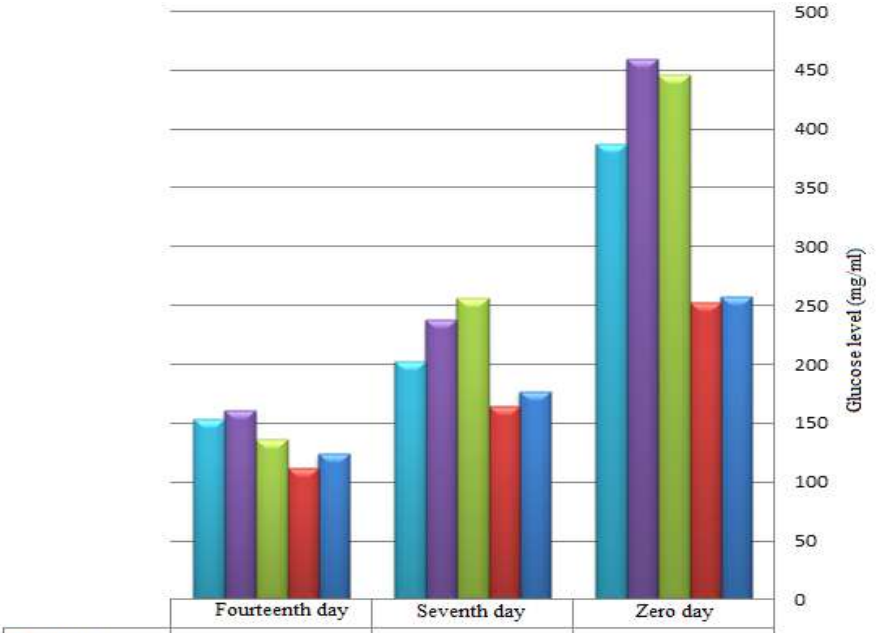
	Fourteenth day	Seventh day	Zero day
Rat 1	62	102	485
Rat 2	75	128	462
Rat 3	76	110	528
Rat 4	63	126	504
Rat 5	65	179	488

Fig. 1 Diabetic rats treated by walnut oil



	Fourteenth day	Seventh day	Zero day
Rat 1	115	183	433
Rat 2	91	110	467
Rat 3	126	171	332
Rat 4	117	121	410
Rat 5	60	193	558

Fig. 2 Diabetic rats treated by walnut septum



	Fourteenth day	Seventh day	Zero day
Rat 1	124	177	257
Rat 2	112	164	253
Rat 3	136	256	446
Rat 4	161	238	459
Rat 5	153	203	387

Fig. 3 Diabetic rats treated by walnut leave aqueous extract

The principle steps of statistically designed experiments are the choice of the experimental design type, determination of response variables, factors and their levels and statistical analysis of the data. In this study

factorial method was employed with the aid of MstatC statistical software to design of experiments. Basically, factorial designs are used to determine which factors are significant and screen them. Two parameters including group (normal control ,diabetic control , diabetic-walnut oil ,diabetic-walnut septum, diabetic- walnut leaves aqueous extract) and time in three level (before alloxan injection, after 7 days of alloxan injection, after 14 days of alloxan injection) were selected to examine the factors affecting diabetes. All factors were examined in various levels and experiments were conducted 5 times to minimize errors. A total of 75 experiments were conducted. The matrix of the factorial design including the Adjusted Sum of Squares (AdjSS) and Adjusted Mean Square (AdjMS) of each factor, P-value and the F-value are described in Table 1.

Table 1 Obtained results from analysis of variance data for selected factorial models

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Factor A	4	1056989.947	*264247.487*	107.5798	0.0000
Factor B	2	536414.107	268207.053	109.1918	0.0000
AB	8	455283.093	56910.387	23.1692	0.0000
Error	60	147377.600	2456.293		
Total	**74**	**2196064.747**			

These values indicate that the models are statistically significant, and there is only less than 0.01% probability that these levels of fit can occur due to random chance [23]. The importance of the data can be judged by its P-value, where values closer to zero denote greater significance. As can be seen the main effects of group (Factor A) and time (factor B) are significant. Also Table 1 reveals that the interaction impact of parameters is influential on diabetes.

Results showed that continuous treatment with walnut oil, septum and leaves aqueous extract for a period of 14 days produced a significant reduction in blood glucose of rats. The blood glucose rate obtained indicated that the extract of walnut oil produces a more significant effect in alloxan –induced diabetic rats than other section of walnut.

The mean blood glucose levels of all groups before intervention, is shown in Figure 4. This Fig reveal that there is significant differences in blood glucose level of control group with other groups ($p<0.05$).

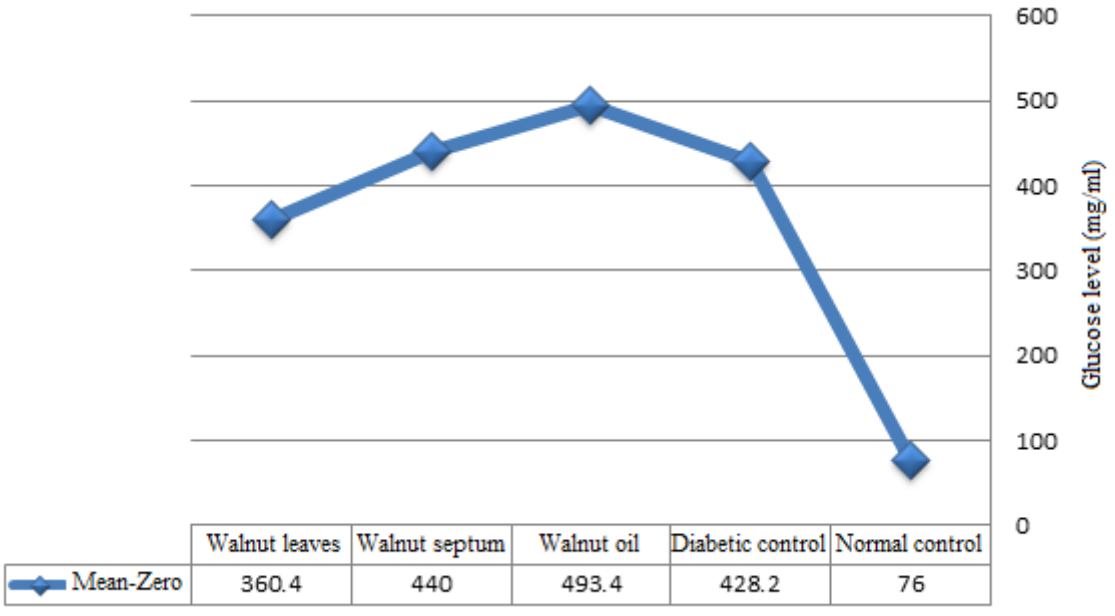


Fig.4 The mean blood glucose levels of all groups before intervention

Results showed that the mean blood glucose levels of control and test diabetic rats after oral administration of walnut oil, septum and leaves modified by therapeutic intervention by 7 and 14 days and the difference between these groups was significant (P<0.05)(Fig. 5-6).

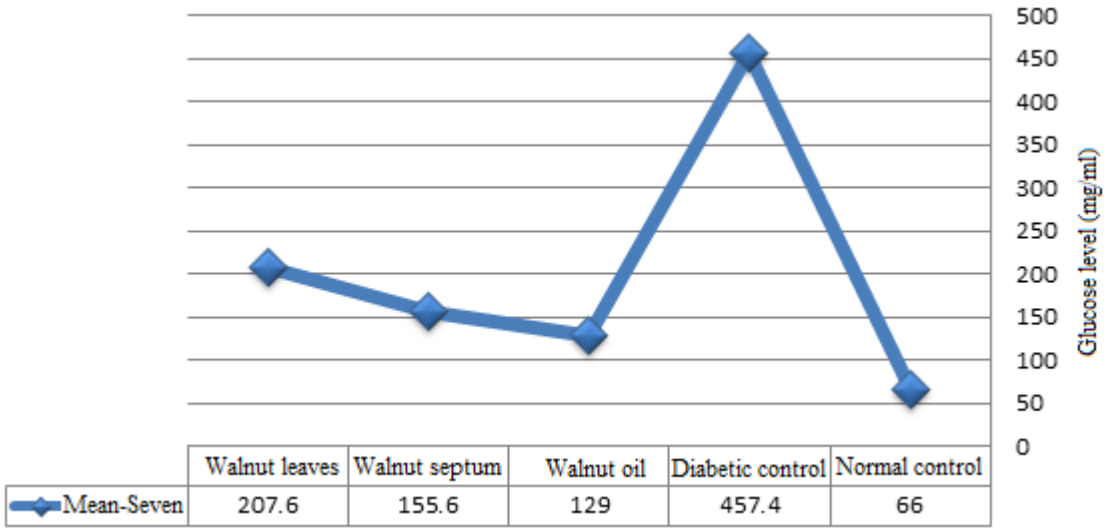


Fig.5 The mean blood glucose levels of all groups after 7 days of intervention

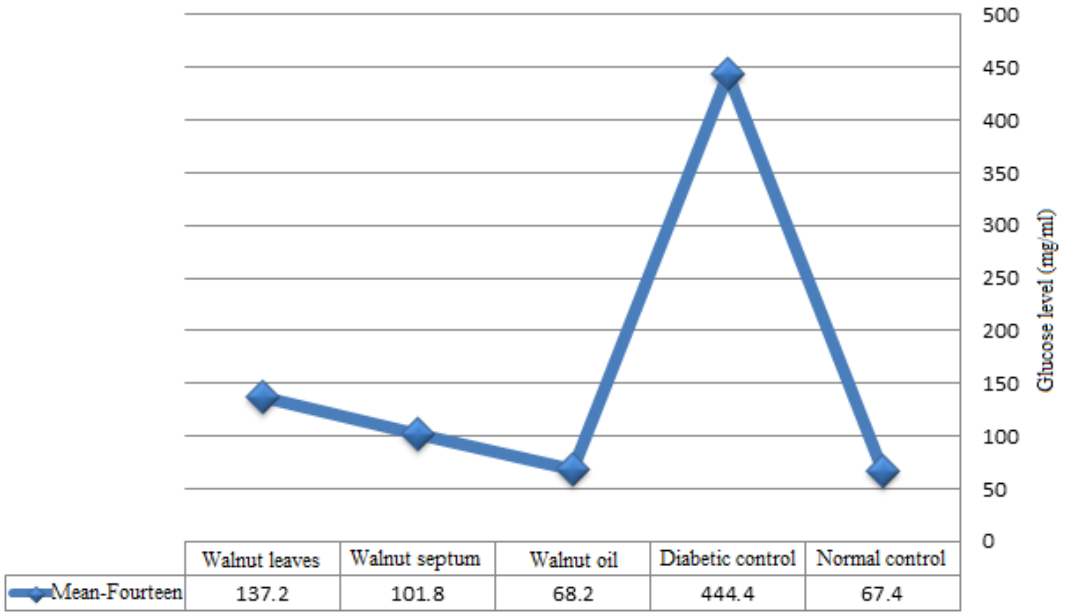


Fig.6 The mean blood glucose levels of all groups after 14 days of intervention

There are the significant difference between mean blood glucose levels of all groups at three different times (before, after 7 and 14 days of intervention)(Fig.7).

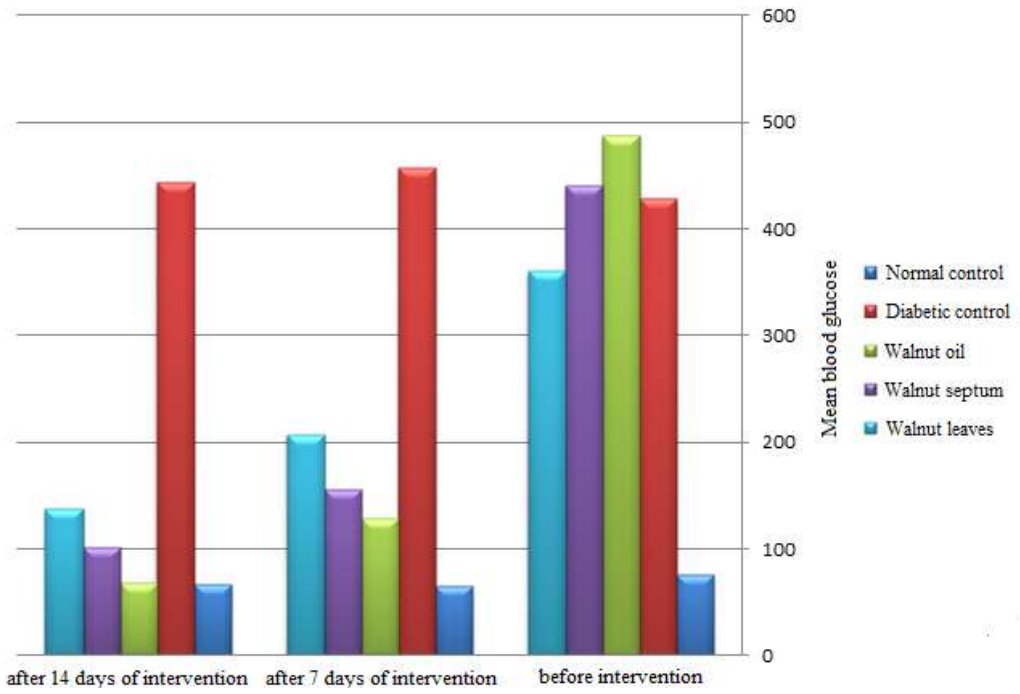


Fig. 7 Comparison between the blood glucose of all groups at three different times

Results showed that blood glucose has been moderated with continuity of using walnut oil, septum and leaves extract. Also hypoglycemia was more than the first week. Long-term effects of these extracts on blood glucose levels are higher than short-term effects. On the other hand they had time-dependent effect on blood glucose level.It has been noted that walnut has a higher antioxidant capacity when compared with other nuts. These antioxidants are possibly of phenolic compounds, including hydrolyzed tannins, tocopherol, and melatonin; all of which have a high antioxidant capacity. Antioxidant has important role in treatment and improvement of glucose homeostasis in patients with diabetes [4].Walnut leaves constitute a good source of antioxidant compounds, namely phenolics, suggesting that it could be useful in prevention of diseases in which free radicals are implicated. Phenolic acid and flavonoid are two major groups of phenolic compounds in walnut leaves. The most important phenolic acid in walnut leaf is caffeoylquinic acid and the main flavonoid is quercetin[3].

Septum of walnut did not effect on the β-cells regeneration. Therefore, this mechanism might have been due to the increase of insulin from remnant β-cells [24-25].It might facilitate utilization of glucose by aninsulin dependent glucose transporter [26]. It seems, continuous consumption of aqueous extract of septum of walnut reduce blood sugar but cannot play a role on repairing beta-cells of langerhan's island. It may be useful for diabetic patients through reducing blood glucose [27].

Conclusion

Regulation of blood glucose concentration plays an important role in diabetic patient. In this paper the effects of walnut oil, septum and leaves aqueous extract were tested on blood glucose in diabetic rats. Results showed that using walnut led had to significant decrease in blood glucose is diabetic rats and they had time-dependent effect on blood glucose level. Alloxan increased oxidative stress in diabetes through free radical generation. So, it needs to explore methods for oxidative damage protection. Hypoglycemic effects of walnut proved blood glucose level by phenolic compounds. Despite the better effect of walnut oil on blood glucose to septum and leaves extract, it can be used as complement for traditional anti-diabetic drugs.

References

1. Williams, G. & Pickup, J. C. *Handbook of Diabetes*. 3rd ed. Oxford, Blackwell Publishing, 2004.pp.48-60.
2. www.livestrong.com
3. Rahimi, P., Kabiri1, N.,Asgary, S.,Setorki, M.,(2011). Anti-diabetic effects of walnut oil on alloxan-induced diabetic rats. *African Journal of Pharmacy and Pharmacology*,5, 2655-2661.
4. ZibaeeNezhad, M.J., Aghasadeghi, K., Hakimi, H., Yarmohammadi, H., Nikaein, F.,(2016). The Effect of Walnut Oil Consumption on Blood Sugar in Patients With Diabetes Mellitus Type 2, *International journal of Endocrinology and Metabolism* , inpress.
5. Hajikhani, R., Solati, J.,(2010).Effects of Walnut Alcoholic Extract (JuglansRegia L.) Septum on Serum Glucose, Insulin and Activities of Aminotransferase Enzymes, *Journal of Applied Chemical Research*, 12, 17-23.
6. Rana M, Dhamija H, Prashar B, Sharma S. Ricinuscommunis L. A Review.International Journal of PharmTech Research.2012;4(4):1706–11.
7. Dehghani,F.,Mashhody,T.,Panjehshahin,M., (2012).Effect of Aqueous Extract of Walnut Septum on Blood Glucose and Pancreatic Structure in Streptozotocin-Induced Diabetic Mouse.*Iranian journal of pharmacology and therapeutics*, 11, 10-14.
8. Zhang, Z., Liao, L., Moore, J., Wu, T., Wang, Z.,(2008). Antioxidant phenolic compounds from walnut kernels (Juglansregia L.),*Food Chemistry*, 113, 160-65.
9. Tapsell, L.C., Gillen, L.J., Patch, C.S., Batterham, M., Owen, A., Bare, M., Kennedy, M.,(2004). Including walnuts in a lowfat/modified-fat diet improves HDL cholesterol-to-totalcholesterol ratios in patients with type 2 diabetes. *Diabetes Care.*27,2777-2783.
10. Zhao, G., Etherton, T.D., Martin, K.R., West, S.G., Gillie , P.J., Kris-Etherton, P.M.,(2004), Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women,*Journal*of *Nutrition*, 134, 2991-2997.
11. Ros E, Nunez, I., Perez-Heras, A., Serra, M., Gilabert, R., Casals, E., Deulofeu, R..,(2004), A walnut diet improves endothelial function in hypercholesterolemic subjects: A randomized crossover trial,Circulation,American Heart Association. 109, 1609-1614.
12. Franz, M.J., Bantle, J.P., Beebe, C.A., Brunzell, J.D., Chiasson, J.L., Garg, A., Holzmeister, L.A., Hoogwerf, B., Mayer- Davis, E., Mooradian, A.D., Purnell, J.Q., Wheeler, M.,(2002), American Diabetes Association. Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications,*Diabetes Care*, 25,148-198.
13. May, Y., Njike, V.Y,, Mille, T.J., Dutta.S., Doughty, K., Treu, J., Katz, D.L. (2010).Effects of Walnut Consumption on Endothelial Function in Type 2 Diabetic Subjects,*Diabetes Care*, 33, 227-232.
14. Ma, Y., Njike, V.Y., Millet, J., Dutta,S., Doughty, K., Treu, J.A.,(2010), Effects of walnut consumption on endothelial function in type 2 diabetic subjects: a randomized controlled crossover tria,*Diabetes Care.*, 33, 227–232.
15. Zargari, A., Therapeutical Plants. 4th ed. Tehran: Tehran University Press; 1989.
16. Fathiazad, F., Garjani, A., Motavalliannaini, A.,(2006), Study of hypoglycemic activity of the hydroalcholic extract of juglansregia in normal and diabetic rats. *Pharmaceutical Sciences*.2,13-17.
17. Ziyyat, A., Legssyer, A, Mekhfi, H., Dassouli,A., Serhrouchni, M., Benjelloun, W.,(1997),Phytotherapy of hypertension and diabetes in oriental Morocco. *Journal of Ethnopharmacology*, 58, 45-54.
18. Jariyah, Azkiyah, L., Widjanarko, S.B., Estiasih, T., Yuwono, S.S. &Yunianta. (2013). Hypocholesterolemic effect of Pedada (Sonneratiacaseolaris) fruit flour in wistar rats. International Journal of Pharm Tech Research,5(4), 1619-1627.
19. NasdiwatyDaud, Rosidah, M PandapotanNasution(2016), Antidiabetic Activity of Ipomoea batatas L. Leaves Extract In Streptozotocin-Induced Diabetic Mice, International Journal of PharmTech Research, (2016), Vol.9, No.3, pp 167-170.
20. Jelodar, GH, Maleki, M., Shahram, S.(2007), Effect of walnut leaf, coriander and pomegranate on blood glucose and histopathologigy of pancreas of alloxan-induced diabetic rats. *African Journal of Traditional, Complementary and Alternative medicines*,4, 299-305.
21. Jariyah, Simon BambangWidjanarko, Yunianta, TetiEstiasih, Hypoglycemic effect of Pedada (Sonneratiacaseolaris) Fruit Flour (PFF) in alloxan-induced diabetic rats, International Journal of PharmTech Research,(2015),7,31-40.

22. Farzami, B., Ahmadvand, D., Vardasbi, S., Majin, F. J. , Khaghani, S.,(2003), Induction of insulin secretion by a component of Urticadioica leave extract in perifused Islets of Langerhans and its in vivo effects in normal and streptozotocin diabetic rats, *Journal of Ethnopharmacology*, 89, 47–53.
23. Gheshlaghi, R. (2007),*Optimization of recombinant protein.*phd thesis,university of waterloo, waterloo.
24. Sharma, S.B., Nasir, A., Prabhu, K.M., Murthy, P.S.,(2006),Antihyperglycemic effect of the fruit-pulp of Eugenia jambolana in experimental diabetes mellitus,*Journal of Ethnopharmacology*,104,367-73.
25. Esmaeili, M.A., Yazdanparas, R., (2004), Hypoglycemiceffect of Teucriumpoliumstudies with rat pancreatic islets, *Journal of Ethnopharmacology*, 95,27-30.
26. Obatomi, D.K., Bikomo, E.O., Temple, V.J.,(1994), Antidiabetic properties of the African mistletoe in streptozotocin-induced diabetic Rats, *Journal of Ethnopharmacology*, 43,13-7.
27. Rahmatullah, M., Azam, Md.N.K., Pramanik, S., Sania, Rahman, S. &Jahan, R. (2012). Antihyperglycemic activity evaluation of rhizomes of Curcuma zedoaria (Christm.)Roscoe and fruits ofSonneratiacaseolaris (L.) Engl. International Journal of PharmTech Research, 4(1):125-129.

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