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Full Length Research Paper

Anti-diabetic effects of walnut oil on alloxan-induced diabetic rats

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Diabetes arises from a deficient production of insulin by the ß-cells of the pancreatic islets. Many traditional treatments have been recommended in the alternative system of medicine for treatment of diabetes mellitus; however, the mechanism of most of the herbs used has not been defined. This study was carried out to clarify the effect of walnut (*Juglans regia*) oil on blood glucose and their possible effect on pancreatic tissue. Diabetes mellitus was induced in 20 adult male rats, using intraperitoneal injection of 75 mg/kg body weight (BW) alloxan. The diabetic rats were divided into two groups; the first group (positive control) received an ordinary diet, and the second group, diabetic rats treated with walnut oil. The non-diabetic rats (negative control group) received neither alloxan nor the oil. Following consumption of oil, blood glucose was measured and on the last day the pancreas were removed and stained with hematoxylin and eosin (H&E), and morphology of the pancreatic sections was studied. The results of this study indicate that walnut oil was able to reduce blood glucose significantly when compared with the control group ($P < 0.05$). In the control, positive group average area of islets in pancreatic sections was significantly reduced in comparison with the control negative (normal health) and diabetic treated with walnut oil ($P < 0.05$). These results suggest the validity of the clinical use of walnut oil in the treatment of diabetes mellitus type I.

Key words: Walnut, alloxan, diabetes mellitus type I, rat.

INTRODUCTION

Diabetes and its complications are major causes of morbidity and mortality in the U.S. and contribute substantially to health care costs (Centers for Disease Control and Prevention). These data have shown that older age, family history and obesity, are strong and consistent determinants of type 2 diabetes (Knowler et al., 1981; King et al., 1984; Haffner et al., 1991). Physiological data show that peripheral insulin resistance and pancreatic β-cell dysfunction are precursors and key determinants of type 2 diabetes (Lillioja et al., 1993). The factors involved to the development of diabetes is changes in sex steroid hormones. However, the mechanisms responsible for these associations as well as the link to sexual dysfunction are not well understood. Imbalances in sex steroid hormone levels are strongly associated with diabetes and this may negatively impact upon sexual function (Kim, 2009). Diabetes mellitus is a group of metabolic disorders characterized by elevation of blood glucose concentration and 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 (Shivananda and Geetha, 2005). 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 (DM) (Nielsen, 1986; Dunger et al., 1996). The positive effects of camel milk on collagen could be explained by its effects on glucose levels and

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insulin action. Camel milk has been shown to normalize insulin action and improve insulin sensitivity (Agrawal et al., 2004) in diabetic rats. The hypoglycemic activity of camel milk may be because of high concentrations of insulin like protein in camel milk which contains about 45 to 128 units/L (Singh, 2001) and also it contains high amount of zinc (Mohamed et al., 1995). Zinc is playing a major role for insulin secretory activity in pancreatic beta cells. in streptozotocin-diabetic rats (Al-Numair et al., 2011). Experiments on rats with streptozotocin-induced diabetes, provided evidence that resveratrol (3,5,4-trihydroxystilbene)-a naturally occurring phytoalexin present in numerous plant species exerts beneficial effects in the organism and may be helpful in preventing and treating some metabolic diseases, including diabetes (Fr ojd o et al., 2008; Szkudelska and Szkudelski, 2010) and induced to decrease blood glucose (Szkudelski and Szkudelska, 2011). Kumar et al. (2011) reported after 60 days of feeding, fish oil diet group had a 17.7% decrease in fasting blood glucose, suggesting overall protection of fish oil from hyperglycemia (Kumar et al., 2011). Flachs et al. (2006) reported a 4% decrease in blood glucose in mice fed with fish oil diet. Walnuts, the seeds of *Juglans regia* L. (Juglandaceae), are a highly nutritious food (Martínez et al., 2010). They are also used as a traditional therapy for treating cough, stomach ache (Perry, 1980) and cancer in Asia and Europe (Duke, 1989). Walnut fat is mostly unsaturated fatty acids, such as linoleic and oleic acid, which may give walnuts additional antiatherogenic properties (Fukuda et al., 2003; Feldman, 2002). Walnuts are highly enriched in omega-6 and omega-3 polyunsaturated fatty acids (PUFA), which are essential dietary fatty acids. Epidemiological and clinical trials suggest that omega-3 PUFA might have a significant role in the prevention of coronary heart disease (CHD) (Harper and Jacobson, 2001; Bucher et al., 2002). Walnut consumption improves endothelial function in type 2 diabetic individuals and may therefore help reduce cardiovascular disease risk in this high-risk population (May et al., 2010). Glibenclamide is an hypoglycemic agent which is also used in researches as a standard medication in the treatment of diabetes (Dhandapani, 2002). At the end, treatment with glibenclamide and walnut oil will be compared.

This study assessed the effect of walnut oil, on blood glucose and some other biochemical parameters in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Preparation of walnut oil

Walnuts used in this experiment were collected in June, 2005 from gardens in Baghbadoran, Isfahan. They were identified and a voucher specimen was deposited at the Herbarium of the Department of Biology, Faculty of Science, Isfahan University (voucher no. 2041), and its oil fraction was extracted with hexane.

Animal treatments and induction of diabetes

Twenty four male Wistar rats, 4 to 5 weeks old, weighing about 150 to 200 g, obtained from Pasteur Institute, Tehran. They were kept under the care of experienced animal technicians. The animals were housed in cages at 24°C, with a 12 h light-dark cycle. The animals were given a standard rat chow diet.

Experimental diabetes was induced by administration of alloxan monohydrate (75 mg/kg of body weight) 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 a diabetic 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 (El–demerdash et al., 2005; Kulkarni et al., 2002; Ragavan and Krishnakumari, 2006). The study was reviewed and approved by the Ethics Committee of Isfahan University of Medical Sciences.

The rats were randomly divided into four groups (n = 6): normal control (non-diabetic control rats given normal saline intraperitoneally, daily for six weeks), diabetic control (untreated diabetic control rats given normal saline intraperitoneally, daily for six weeks to equalize stress induced by injections in all groups), diabetic-walnut oil (diabetic rats given extract of walnut oil intraperitoneally, daily for six weeks) and diabetic-glibenclamide (diabetic rats given glibenclamide 0.6 mg/kg intraperitoneally, daily for six weeks) (Dhandapani et al., 2002).

Blood sample collection and analysis

Blood samples after 16 h of fasting were collected three times: time 0 was before injection of alloxan, time 1 was two weeks after injection of alloxan and time 2 was six weeks after injection of alloxan. Plasma insulin levels were estimated by enzyme-linked immunosorbant (ELISA) method, using the commercially available kit obtained from Boehringer-Mannheim, Germany (Enzymun-test system) on ES 33 analyzer, that is, blood glucose, hemoglobin A1c (HbA1c), total cholesterol (cho), triglyceride (TG) and low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C) and very low density lipoprotein-cholesterol (VLDL-C) levels were measured using special kits (DiaSys, Germany) which utilized the colorimetric method, in an autoanalyzer (Hitachi autoanalyzer, Hitachi Co., Tokyo).

Histological studies

After a 6-week experimental period and the last blood sampling, the whole pancreas was removed after sacrificing the animal and was fixed in 10% formalin for histopathological examination. Sections were cut and stained by hematoxylin and eosin (H&E) for histological examination (Nagappa et al., 2003).

Statistical analysis

All values were expressed as mean ± standard deviation (SD). Significant differences among the groups were determined by one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) 13.0 software package program. Values of $P < 0.05$ were taken as statistically significant.

RESULTS

At time 0

As shown in Table 1, the levels of different biochemical

Table 1. Different biochemical parameters levels in non-diabetic control, diabetic control, diabetic treated rats with walnut oil and diabetic treated rats with glibenclamide (Means ± SE).

Parameter	Time	Non diabetic control	Diabetic control with no treatment	Diabetic treated with walnut oil	Diabetic treated with glibenclamide
Glucose (mg/dl)	0	95.5 ± 13.52	98.5 ± 12.43	96.0 ± 13.21	95.0 ± 3.0
	1	101.25 ± 26.22	492.4 ± 95.56*#	228 ± 22.13#*	240 ± 26.45#*
	2	97.25 ± 12.78	599 ± 82.56#**	86.3 ± 5.07‡	122.33 ± 32.30‡
Insulin (µu/ml)	0	13.52 ± 1.12	13.04 ± 1.26	13.1 ± 1.16	14 ± 1.35
	1	13.52 ± 1.06	5.27 ± 0.91#*	6.1 ± 1.07#*	6.6 ± 0.83#*
	2	12.55 ± 1.35	4.86 ± 1.41#**	14.35 ± 2.73‡	12.5 ± 1.27‡
HbA1c (%)	0	4.22 ± 0.22	4.25 ± 0.25	4.5 ± 0.27	4.03 ± 0.23
	1	4.3 ± 0.16	4.44 ± 0.63	4.1 ± 0.12	4.46 ± 0.66
	2	4.5 ± 0.21	6.9 ± 1.75#**	4.20 ± 0.21	4.16 ± 0.15
LDL-C (mg/dl)	0	14.75 ± 2.5	11.16 ± 2.22	14.26 ± 3.22	13.66 ± 1.52
	1	15.0 ± 1.82	20.2 ± 1.48#*	26.7± 3.05	22 ± 8.88#*
	2	16.25 ± 1.70	33.0 ± 3.36#**‡	21 ± 3.11#**‡	15.66 ± 3.21
HDL-C (mg/dl)	0	37.25 ± 1.70	37.5 ± 1.87	36.5 ± 1.87	37.0 ± 1.0
	1	40.25 ± 10.87	35.1 ± 6.08#	43.2± 5.54	49.0 ± 21.93
	2	41.75 ± 6.39	33.33 ± 6.65#	41.4 ± 5.12	42.33 ± 9.45
VLDL-C (mg/dl)	0	17.45 ± 3.54	16.8 ± 3.32	16.8 ± 3.32	19.26 ± 1.13
	1	15.15 ± 3.04	23.56 ± 11.97#*	16.45 ± 1.25#*	17.66 ± 7.85#
	2	16.55 ± 2.34	30.5 ± 6.6‡	14.14 ± 3.64	14.0 ± 3.12
Cholesterol (mg/dl)	0	61.25 ± 6.29	61.25 ± 6.18	61.5 ± 5.24	62.0 ± 7.21
	1	62.57 ± 3.20	91 ± 14.71#*	96.6 ± 5.50*#	100.0 ± 34.21#
	2	64.5 ± 5.19	127.5 ± 25#**‡	77.0 ± 5.31	60.33 ± 5.50
Triglyceride (mg/dl)	0	87.25 ± 17.72	83.9 ± 16.52	84.0 ± 16.60	96.33 ± 5.68
	1	85.75 ± 15.23	117.8 ± 59.87#*	85.2 ± 6.25	88.33 ± 39.27
	2	82.75 ± 11.70	52.5±33.04#**‡	69.7 ± 16.21	70.0 ± 15.62

*Means differ significantly between time 0 and time 1($P < 0.05$). **Means differ significantly between time 2 and time 0 ($P < 0.05$). #Means differ significantly in comparison with non-diabetic control group ($P < 0.05$). ‡Means differ significantly between time 2 and time 1 ($P < 0.05$).

parameters in serum of all four groups had no meaningful deference.

At time 1

Blood glucose level was found to be significantly increased in diabetic control rats, diabetic rats treated with walnut oil and diabetic rats treated with glibenclamide, and this was associated with decrease in insulin level. Cholesterol, VLDL-C, LDL-C and TG were significantly increased and HDL-C decreased in diabetic control. Cholesterol and VLDL-C were significantly increased in diabetic rats treated with walnut oil, and diabetic rats treated with glibenclamide LDL-C was significantly increased as compared to non diabetic control rats ($P < 0.05$), and also in comparison with time 0 ($P < 0.05$). Insulin significantly decreased at time 1 in all diabetic groups as compared to non diabetic control rats ($P < 0.05$), and also in comparison with time 0 ($P < 0.05$).

At time 2

Treatments with either walnut oil or glibenclamide produced a significant decrease in blood glucose levels along with significant increase in insulin levels in comparison with time

Table 2. Size of islets of Langerhans in four experimental groups.

	Time	Non diabetic control	Diabetic control with no treatment	Diabetic treated with walnut oil	Diabetic treated with glibenclamide
Size of Langerhans islets (micron)	2	1.64 ± 0.3	0.62 ± 0.4*	1.02 ± 0.44	1.48 ± 0.6

*Means differ significantly between diabetic control and non-diabetic control group (P < 0.05).

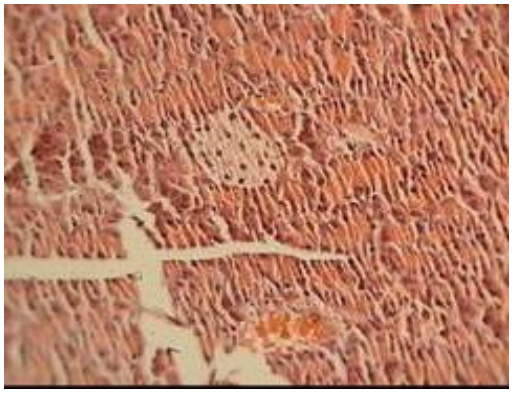

Figure 1. Cross-section of Langerhans islets in diabetic control group.

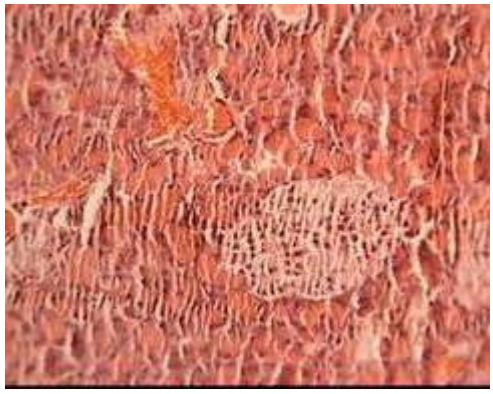

Figure 2. Cross-section of Langerhans islets in non-diabetic group.

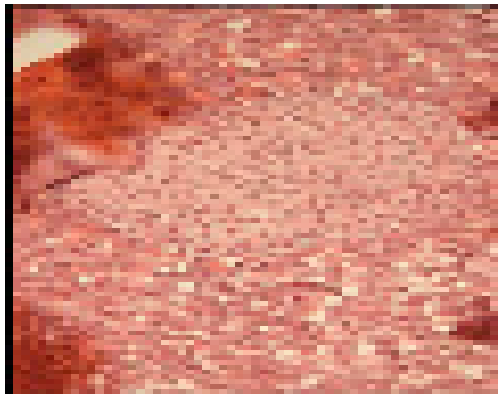

Figure 3. Cross-section of Langerhans islets in diabetic rats treated with walnut oil.

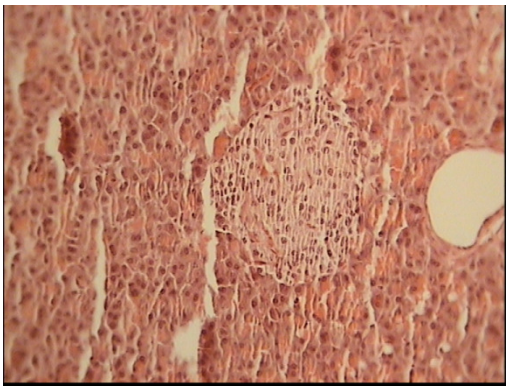

Figure 4. Cross-section of Langerhans islets in diabetic rats treated with glibenclamide.

1. LDL-C levels decreased significantly by walnut oil due to six weeks of treatment (Time 2). HbA1C was found to be significantly higher in diabetic control rats (P < 0.05). In contrast, diabetic rats treated with walnut oil and diabetic rats treated with glibenclamide showed lower mean HbA1C.

Results of the histomorphometric study

Histopathological study of islets of Langerhans revealed that size of Langerhans islets in diabetic control group had a significant difference in comparison with non-diabetic control group (P < 0.05).

There was no significant difference in the size of Langerhans islets between the treated rats (with walnut oil or glibenclamide) and non-diabetic control rats (Table 2 and Figures 1 to 4).

DISCUSSION

This study demonstrated that consumption of walnut oil for six weeks, significantly reduce biochemical parameters (blood glucose, insulin, HbA1C, total cholesterol, LDL-C, VLDL-C, HDL-C, and triglyceride) and size of

Langerhans islets in alloxan-induced diabetic rats and when compared with the effects of glibenclamide. Alloxan and streptozotocin (STZ) are widely used to induce experimental diabetes in animals (Szkudelski, 2001). STZ action in B cells is accompanied by characteristic alterations in blood insulin and glucose concentrations. 2 h after injection, hyperglycemia is observed with a concomitant drop in blood insulin. About 6 h later, hypoglycemia occurs with high levels of blood insulin. Finally, hyperglycemia concentrations reflect abnormal-lities in B cell function. STZ impairs glucose oxidation (Bedoya et al., 1996) and decreases insulin biosynthesis and secretion (Bolaffi et al., 1987; Nukatsuka et al., 1990b). It was observed that STZ at first abolished the B cell response to glucose. Temporary return of responsiveness then appears, which is followed by its permanent loss and cells are damaged (West et al., 1996). Alloxan is rapidly taken up by the b cells, it is not toxic by itself (Wilson et al., 1984). The selective toxicity of alloxan to pancreatic b cells is due to its direct effect on islet cell permeability (Szkudelski, 2001; Watkins et al., 1979) and acts at the site of hexose transport as it inhibits glucose-stimulated insulin release (Robertson et al., 1988) and generates free radicals (Houee et al., 1981) that are toxic to various cellular constituents. It showed that significant decrease in blood glucose and elevated insulin level when STZ-diabetic rats were administered amaranth grain and its oil fraction (Kim et al., 2006). Walnut leaves constitute a good source of anti-oxidant 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 (Fukuda, 2003; Pereira et al., 2007; Solar et al., 2006). Some studies have shown that flavonoids are able to decrease plasma glucose level (Li, et al., 2004). Quercetin inhibits glucose transporter (GLUT2), so diminishes glucose intestinal absorption (Kwon, 2007). Caffeoylquinic acid (chlorogenic acid) is a specific inhibitor of glucose-6-phosphate translocase and reduces hepatic glucose production (Hemmerle, 1997), thus decrease blood glucose level and HbA1C (Dhandapani, 2002). Plant antioxidants are able to restore and re-generate pancreatic B cells. The results from the studies on garlic, onion and fenugreek show that in diabetic rats treated with anti-oxidants the number of Langerhans islets has increased significantly (El–demerdash, 2005; Jelodar, 2005). Walnuts also contain several phytosterols, as it appears that they can inhibit intestinal absorption of cholesterol (Amaral et al., 2003). Fatty acid composition can influence various physiological and bio-chemical processes, including blood pressure regulation, glucose metabolism, lipid metabolism, platelet aggregation and erythrocyte deformability (Wright et al., 1988). Studies to evaluate that oils rich in v-3 and v-6 long-chain fatty acids can significantly attenuate alloxan-induced diabetes in animals. These oils are able to inhibit or reduce the free radicals and thus prevent diabetes (Mohan and Das, 2001; Suresh and Das, 2006). Mohan and Das (2001) showed that PUFA-rich oils prevented alloxan-induced damage to the pancreatic b cells. Their ability to enhance antioxidant status, suppress cytokine production (Chew et al., 1997; Suresh and Das, 2001; Mohan and Das, 2001; Suresh and Das, 2003a; Suresh and Das, 2003b). Polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) have received a lot of attention in the last decade and their health benefits are becoming increasingly evident (Jimenez-Gomez et al., 2008; Micallef and Garg, 2009; Ouellet et al., 2008). Oleic acid increase insulin secretion in the presence of the inflammatory cytokine TNF-α. The mechanisms of the anti-diabetogenic action of oleic acid are very likely multi-faceted. The role of oleic acid in the presence of the proinflammatory cytokine TNF-α is clear. Dietary fatty acid intake, particularly unsaturated fatty acids, may also alter the composition of membrane phospholipids in addition to binding nuclear transcription factors. Changes in phospholipid composition may in turn change membrane fluidity; thereby, altering the binding of cytokines to their corresponding receptors. Arachidonic acidis is effective in preventing alloxan-induced toxicity to b cells *in vivo*, the ability of arachidonic acidis to prevent the development of diabetes induced by alloxan may be due to decreased production of free radicals and lipid peroxides (Laight et al., 1998; Das, 2001). Studies were also conducted specifically on walnuts, findings show that frequent consumption of moderate quantities of walnuts favorably modified the lipoprotein profile and decreased serum levels of total cholesterol (Houseknecht et al., 1998; Higa et al., 1999). Nuts are also rich in phytosterols, which due to their structural similarity with cholesterol, inhibit its intestinal absorption, thereby lowering total plasma cholesterol and LDL levels (Ha et al., 1990; Fukuzawa et al., 1999). The results of the present study suggest that walnut oil is effective in preventing alloxan-induced on biochemical parameters like blood glucose, insulin level, HbA1C, cholesterol, triglyceride, LDL-C, HDL-C and VLDL-C in diabetic rats.

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