



Effects of safflower oil on FBS and lipid profile in alloxan induced diabetic rats and its mechanism

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ABSTRACT

Background & Aim: Diabetes mellitus often has been referred to as a syndrome of disordered metabolism, usually due to a combination of hereditary and environmental causes, resulting in abnormal of high blood sugar levels (hyperglycemia). Nowadays, no-drug treatments (medicinal plants) are novel therapeutic approaches in the treatment of diabetes.

Experimental: This study aimed at assessing the effect of *Carthamus tinctorius L* oil on the blood glucose and lipid profile in diabetic rats. Eighteen male rats were divided into 3 groups as follows: control, diabetes treated with glibenclamide and diabetes treated with 200 mg/kg b.wt safflower oil. Diabetes was induced in rats by intraperitoneal administration of single dose of alloxan monohydrate (120 mg/kg body weight). The oil of (*Carthamus tinctorius L.*) at a dose of 200 mg/kg b.wt was administered every other day to the diabetic rats for a period of 30 days.

Results: The results show that the safflower oil exhibited significant hypoglycemic and hypolipidemic effect in hyperglycemic rats.

Industrial and practical recommendations: Phenolic compounds and free fatty acids in safflower oil play an important role in regulating animal insulin secretion response and glucose homeostasis.

1. Introduction

Diabetes mellitus is a group of metabolic alterations characterized by hyperglycemia resulting from defects in insulin secretion, action or both. It has already been established that chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels (Olatunde et al., 2014). The incidence of diabetes has increased worldwide in recent years. The estimated number of

people with diabetes was 30 million in 1985, 150 million in 2000 and then 246 million in 2007, according to the International Diabetes Federation. It expects this number to hit 380 million by 2025 (Samreen et al., 2009).

Available treatments for diabetes mellitus are insulin, sulfonylureas, biguanides and glinides. Many of these treatment agents have a number of serious adverse effects such as hypoglycemia, drug-resistance, dropsy, and weight gain (Tahrani et al., 2010; Patel et al., 2012). The use of medicinal plants for the treatment of diabetes mellitus dates back from the Ebers papyrus

of about 1550 B.C. A multitude of herbs, spices and other plant materials have been described for the treatment of diabetes throughout the world (Kaleem *et al.*, 2008). Medicinal plants have gained importance for the treatment of diabetes mellitus. Anti-hyperglycemic activity of most medicinal plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output, inhibit the intestinal absorption of glucose or facilitating metabolites in insulin dependent processes (Malviya *et al.*, 2010). Many studies have demonstrated evidence of the health benefits of natural products. Plant extracts have been tested on a variety of physiological disorders, including diabetes mellitus. Studies have tested aqueous extracts, plant fractions extracts, families of active compounds, and specific active compounds such as flavonoids (Sasidharan *et al.*, 2011). It seems that many vegetable oils are really interesting and can be used in the improvement of human health, particularly, to prevent or to treat diabetes mellitus complications (Berraouan *et al.*, 2013).

Safflower (*Carthamus tinctorius* L.) is a multipurpose oilseed crop grown mainly for production of high quality edible oil rich in polyunsaturated acids (Asgarpanah *et al.*, 2013). For a long time *C. tinctorius* L. has been used in traditional medicines as a purgative, analgesic, antipyretic and an antidote to poisoning. It is a useful plant in painful menstrual problems, post-partum hemorrhage and osteoporosis. *C. tinctorius* L. has recently been shown to have antioxidant, analgesic, anti-inflammatory and antidiabetic activities (Louei Monfared *et al.*, 2013). Safflower (*Carthamus tinctorius* L.) has a long history of cultivation as an oilseed crop and as a source of red dye (carthamin). Carthamin is extracted from its flowers and it is used for treatment in the form of infusion, for circulatory system related diseases (Asgarpanah *et al.*, 2013). Traditionally, the concept of oil quality is almost exclusively associated with the fatty acid composition of the oil. The only essential fatty acid in human nutrition is linoleic acid. Some nutritionists also believe that the omega-3 fatty acids are essential. Linoleic acid (18:2) is "polyunsaturated" and has a high value for unsaturated fatty acids. In recent years, especially in the developed countries, safflower has attracted significant attention due to its

edibility and medicinal values (Arslan *et al.*, 2005; Stephen *et al.*, 2010).

2. Materials and Methods

2.1. Preparation of safflower oil

The seed of safflower was collected during September 2006 from Isfahan province. The plant was identified by Dr. L. Ghaem Maghami from Isfahan University of Sciences and a voucher specimen No.2338 kept in the herbarium of the Sciences Faculty. Five percent v/v of the resulting oil was prepared, using saline solution of Dimethyl sulfoxide (DMSO) (Lahlou, 2004).

2.2. Experimental animals

Eighteen 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. All animal handling and manipulation procedures were performed according to the guideline of the Animal Welfare Act and Office of Research Ethics Committee of University of Shahrekord approved the experimental protocols.

2.3. Induction of experimental diabetes

After fasting for 18 h, animals in the diabetic groups were subjected to a single intraperitoneal injection of 120 mg/kg body weight alloxan monohydrate (Sigma – Germany) dissolved in sterile distilled water. 48 h after alloxan injection, fasting blood glucose (FBG) was determined using glucometer. Rats showing glucose concentration above 200 mg/dl were considered diabetic (Maithili *et al.*, 2011).

2.4. Experimental Design

Eighteen male rats were divided into 3 groups as follows: control, diabetes treated with glibenclamide (Standard group) and diabetes treated with 200 mg/kg b.wt safflower oil (safflower oil group) (Ashraduzzaman *et al.*, 2011). Treatment group was administered with safflower oil at dose of 200 mg/kg body weight by gavage. Standard and control animals

were treated with standard drug glibenclamide (Hakim Pharmacy -Iran) at an oral dose of 1mg/kg body weight and distilled water respectively. All doses were started 48 h after alloxan injection. The above treatments were given once daily for 30 days. On 31 day under ether (Hakim Pharmacy -Iran) anesthesia cardiac blood 2ml was collected for estimation of glucose and lipid profile.

2.5. Statistical analysis

The results were analyzed by using one way ANOVA followed by Dunne's test, $p < 0.01$ was considered to be significant.

3. Results and discussion

3.1. Hypoglycemic effect

Administration of safflower oil produced significant hypoglycemic effect as compared to control group in hyperglycemic animals (Table 1).

3.2. Lipid profile

In hyperglycemic animals safflower oil favorably altered the plasma lipid profile by significantly decreasing total cholesterol, LDL, VLDL and by significantly increasing HDL (Table 2).

Diabetes mellitus is a multifactorial chronic disease and has become a challenging job to treat with currently available drugs due to recurrent drawbacks leading to poor compliance (Lyra *et al.*, 2006). Impaired carbohydrate metabolism is the main metabolic disorder in diabetes mellitus leading to hyperglycemia. Altered digestion and absorption of dietary carbohydrate, depletion of glycogen storage, increased gluconeogenesis and over output hepatic glucose, Beta cell (dys) function and defect in insulin signaling pathways are more important causes of hyperglycemia (Glugliano *et al.*, 2008). Among the known natural bioactive components and phytochemicals, recently polyphenols are very popular because of their anti-hyperglycemic effects, safety and non-side-effects. Potential efficacy of polyphenols on carbohydrate metabolism and glucose homeostasis has been well investigated in *in vitro*, animal models and some clinical trials (Hanhineva *et al.*, 2010). One of the most well-known properties of the polyphenols, especially flavonoids, phenolic acids and tannins, on carbohydrate metabolism is inhibition of α -glucosidase

and α -amylase, the key enzymes responsible for digestion of dietary carbohydrates to glucose (Tadera *et al.*, 2006). *Carthamus tinctorius* commonly known as safflower belongs to the family asteraceae and since centuries it was grown from China to Mediterranean regions, but at presently is grown for commercial purposes in Pakistan, India, USA, Ethiopia, Mexico, Kazakhstan, Argentina, Australia, Spain, Turkey, Canada and Iran (Ziarati *et al.*, 2012).

Table 1. Effect of safflower oil on fasting blood glucose (mg/dl).

Groups	Before treatment	After treatment
Control	322.22 ± 15.56	330.4 ± 21.99
Standard (GLB0.9)	356.02 ± 9.54	234.3 ± 318.35**
Safflower oil	358 ± 31.14	136.4 ± 6.18

ANOVA followed by Dunnet's test, ** $p < 0.01$.

Table 2. Effect of safflower oil on lipid profile in hyperglycemic rats.

Groups	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)
Control	166.6 ± 5.94	96.1 ± 1.15
Standard (GLB0.9)	142 ± 7.27**	85.20 ± 1.04**
safflower oil	109.17 ± 2.5**	46.33 ± 1.22**

Continued table 2. Effect of safflower oil on lipid profile in hyperglycemic rats.

Groups	LD (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
Control	127.7 ± 3.30	12.27 ± 0.49	19.93 ± 0.31
Standard (GLB0.9)	114.8 ± 3.28**	19.13 ± 1.12**	16.15 ± 0.21**
Safflower oil	60.23 ± 4.70**	34.07 ± 1.31**	10.68 ± 0.25**

ANOVA followed by Dunnet's test, ** $p < 0.01$

Many phenolic compounds such as serotonin derivatives, serotonin glycosides, lignin and flavonoids were isolated from *Carthamus tinctorius* L. seeds, and these compounds comprise a large proportion of the phenolic compounds. (Asgarpanah *et al.*, 2013; Seok-Yeong *et al.*, 2013). These compounds have been investigated for various physiological activities such as

anti-cancer, antioxidant and anti-inflammatory effects. Moreover, several flavonoid compounds, such as kaempferol, rutin hydrate, luteolin, baringin, and quercetin hydrate, were detected by HPLC (Yu *et al.*, 2013). Some polyphenols are able to regulate the key pathways of carbohydrate metabolism and hepatic glucose homeostasis including glycolysis, glycogenesis and gluconeogenesis, usually impaired in diabetes (Jung, 2007). The results from the in vitro studies showed that some polyphenolic compounds such as quercetin, 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 (Zhang *et al.*, 2011; Park *et al.*, 2007).

Some polyphenols also have potential to induce phosphatidylinositol 3-kinase (PI3k) as a key signaling pathway for up-regulation of glucose uptake (Kumar *et al.*, 2009). Some of the polyphenolic compounds protect β -cells from hyperglycemia-induced and oxidative induced damage; oral administration of phenolic-rich chestnut extract in STZ-induced diabetic rats had favorable effects on serum glucose and viability of β -cell through attenuation of oxidative stress, enhancing the natural antioxidant system, and inhibition of lipid peroxidation (Yin *et al.*, 2011). Some protective effects of polyphenols on β -cells are related to the ability to modulate key cellular signaling pathways; anthocyanin-rich Chinese bayberry extract showed protective effects for pancreatic β cells against oxidative damage through up-regulation of heme oxygenase-1, modulation of ERK1/2 and PI3K/Akt signaling path way and inhibition of β cells apoptosis (Zhang *et al.*, 2011).

Dyslipidemia, undesirable changes in vascular endothelial and smooth muscle cells, lipid peroxidation especially oxidized low density lipoprotein particles, oxidative damage and increased inflammatory mediators including chemokines and cytokines, hypercoagulation and platelet activation have been considered as the main metabolic abnormalities in diabetes mellitus leading to cardiovascular disease (Thomas *et al.*, 2007). There is growing evidence suggesting that dietary intake of polyphenol-rich foods and supplementation with these bioactive components could have protective effects against diabetes-induced cardiovascular pathogenesis; the mechanisms involved

in these properties mainly include regulation of lipid metabolism, attenuation of oxidative damage and scavenging of free radicals, improvement of the endothelial function and vascular tone, enhancement the production of vasodilation factors such as nitric oxides and inhibition the synthesis of vasoconstrictors such as endothelin-1 in endothelial cells (Lecour *et al.*, 2011; Stoclet *et al.*, 2004). For safflower oils analyzed total based on fatty acids, the proportion of saturated fatty acids (SFA) slightly varies between 10.76% and 9.67%, the unsaturated fatty acids (UFA) varies between 90.2% and 89.23%. Safflower seed oils analyzed show that palmitic, oleic, stearic and linoleic esters are the main FAME identified. Palmitic acid was the major saturated fatty acid (7.2-8.6%) followed by stearic acid (2-2.39%). Linoleic acid is the principal fatty acid (77.94-79.49%) followed by oleic acid as the second main fatty acid (9.5-11.29%). The relative proportions of these two major fatty acids determine relevant technological and nutritional properties of edible oil (Rivellese *et al.*, 2003; Ibrahim *et al.*, 2005; Stephanie *et al.*, 2014).

Free fatty acids play an important role in regulating animal insulin secretion response. Acute elevated free fatty acids increased animal insulin secretion and glucose-stimulated insulin secretion. Linoleic acid is a polyunsaturated essential fatty acid called omega-6 fatty acid (Pitts *et al.*, 2003). There is evidence that free fatty acids play an important role in regulating animal insulin secretion response and glucose homeostasis. It was recognized in a previous study that elevated plasma free fatty acids had both stimulatory and inhibitory effects on insulin secretion (Lai *et al.*, 2013). Elevated free fatty acids were proved to enhance glucose-stimulated insulin secretion in fasted rats. Prolonged exposure to elevated fatty acids induced an impairment of animal insulin secretion in β -cells secretion function, whereas an acute exposure was found to enhance insulin secretion. In addition, saturated fatty acids induced lip apoptosis in human β -cells, whereas unsaturated fatty acids had no effect (Gravena *et al.*, 2002). Insulin tropic effect of free fatty acids was profoundly influenced by the chain length and degree of saturation of individual fatty acids under certain circumstances. Long-chain and saturated fatty acids were more effective than medium-chain and unsaturated fatty acids (Rivellese *et al.*, 2003).

4. Conclusions

This study clearly shows that the safflower oil has shown a significant anti-hyperglycemic effect which may be attributed to the presence of phenolic compounds and fatty acids with insulin tropic effect. Phenolic compounds and free fatty acids in safflower oil play an important role in regulating animal insulin secretion response and glucose homeostasis.

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