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Full Length Research Paper

Effects of hydromethanolic extract of saffron (*Crocus sativus*) on serum glucose, insulin and cholesterol levels in healthy male rats

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Saffron and its constituents are reported to have a wide range of biological activities. To determine the hypoglycemic effect of hydromethanolic extract of saffron, which has rarely been dealt with in previous studies and hypolipidemic effect of saffron extract, we investigated the effects of hydromethanolic extract of saffron on healthy male rats. The present study also aimed at evaluating the effects of saffron extract on insulin secretory function of pancreatic β-cells. In this study, healthy male rats (n = 30) were divided into 3 groups of ten: The test group (saffron group), the sham group (physiologic serum group) and the normal group. The test group received 50 mg/kg saffron extract through intraperitonal injection. The sham group was also intraperitoneally injected by 50 mg/kg of physiologic serum solvent for 2 weeks. Daily injection was repeated at 10 am. Blood samples were obtained from retro-orbital sinus before administration and on the 7th and 14th days of administration. The serum glucose and cholesterol levels were measured by the enzymatic method and insulin levels were measured using insulin kit by ELISA method. The results showed that hydromethanolic extract of saffron on the 7th day of administration, significantly decreased serum glucose in saffron group without having any effects on serum cholesterol and insulin levels. On the 14th day of administration, we found significant decrease of serum glucose and cholesterol levels in saffron group. In addition, after 2 weeks, serum insulin in test group significantly increased compared to the sham and normal groups. No changes were observed on serum glucose, cholesterol, and insulin levels in sham and normal groups on the 7th and 14th days of administration. These results suggest that saffron extract has hypoglycemic and hypolipidemic effects on healthy male rats. Moreover, saffron extract could increase their insulin secretion from pancreatic β-cells. Therefore, it should be considered in future therapeutic researches.

Key words: Saffron, insulin, cholesterol, glucose, healthy male rat.

INTRODUCTION

Saffron is the dried stigmata of the flowers of the saffron (*Crocus sativus* L., Iridaceae). Saffron has various pharmacological effects and is regarded as a potent drug. Thus research on the biological activities of saffron and its active constituents may have clinical and public health application. The main active constituents of this plant are crocetin, safranal, picrocrocin and crocins (Trantilis et al., 1995). Saffron is used in folk medicine as an antica-tarrhal, antispasmodic, gingival sedative, nerve sedative, carminative, diaphoretic and expectorant (Riose et al., 1996).

In modern pharmacological studies, saffron or its active constituents has demonstrated anti- inflammatory (Hosseinzadeh et al., 2002), anti-oxidant (Chen et al., 2008; Kanakis et al., 2007), hypolipidemic (Sheng et al.,

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2006), insulin resistance reducing (Xi et al., 2005), tissues oxygenation enhancing (Gainer et al., 1993) and hypoglycemic (Mohajeri et al., 2009) effects. Recently, it was found that saffron extract, crocin and safranal exhibited significant radical scavenging activity and thus antioxidant activity (Assimopoulou et al., 2005). Regarding the hypolipidemic effects of saffron and its active constituents, Sheng et al. (2006) indicated that crocin has lipid lowering properties and selectively inhibits the activity of pancreatic lipase as a competitive inhibitor. Moreover, in another study we found that crocin has a potent hypotriglyceridemic and hypocholesterolemic activity in atherosclerotic quails (He et al., 2005). On the other hand, results of absorption and fluorescence studies indicate that crocetin binds strongly to albumin (Miller et al., 1982). In a recent study, in experimental hyperlipidemia rats with 2 months feeding heavy cholesterol, crocin decreased level of cholesterol in rats (Xu et al., 2005). Further studies have verified that cro-cetin could reduce the levels of serum total cholesterol, triglyceride and malondialdehde and inhibit the descending of nitric oxide in serum of hyperlipidamic-diet quails (He et al., 2007). Some investigations indicate that saffron and its constituents affect insulin resistance and insulin secretion from pancreatic β-cells. Recent reports have described that crocetin can enhance insulin sensitivity and favourably regulate TNF-α and adiponectin expression in white adipose tissues in fructose-fed rats (Xi et al., 2007). El- Daly (1998) described that crocus sativus stigmats given together with cisplatin lead to an even greater decrease in blood glucose than that seen with cisplatin alone. In a recent study, Mohajeri et al. (2009) showed that saffron extract significantly decreased blood glucose and increased serum insulin in diabetic rats. However, to date, little attention has been paid to the effects of saffron extract on blood glucose and insulin secretion from pancreatic β-cells. Therefore, the present study aims to assess the effects of hydromethanolic extract of saffron on serum glucose, cholesterol and insulin levels in healthy male rats.

MATERIALS AND METHODS

Plant and preparation of saffron extract

C. sativus L. stigmas were collected from Ghaen (khorasan province, Northeast of Iran). A voucher specimen was authenticated and deposited in the Herbarium of Faculty of Pharmacy, Tabriz University of Medical sciences. In the maceration method 30 g of stigmas powder were macerated in n-hexane and hydromethanolic solution (70%) for 24 h respectively (each 1.5 l). The extracting action was repeated three times. Hydromethanolic extract was evaporated to dryness under reduced pressure at 40°C by rotary evaporator. The yield of the hydromethanolic extract was 56.85% (w/w). Dried extracts were maintained in refrigerator and under freeze.

Animals

Male Sprague–Dawley rats weighing 200 - 250 g were obtained from Pasteur Institute of Iran. Animals were housed in individual cages under standard laboratory conditions in a 12 h/12 h light /dark cycle and at a temperature of 21 - 23°C. Rats had free access to food and water. The animals were randomly divided into three groups, each of which contained 10 rats. Group 1 was the test group (saffron group), group 2 was the sham group (physiologic serum group) and group 3 was the normal group.

Implementation method

On the day before the experiment, blood samples were collected from retro-orbital sinus from three groups. After this, saffron extract (50 mg/kg) was injected into the test group intraperitoneally and the sham group received physiologic serum solvent (50 mg/kg) intraperitoneally. During a period of two weeks, daily injection of saffron extract to test group and physiologic serum solvent to sham group was repeated at 10 am On the 7th and 14th day as the day before treatment, blood samples were collected separately of three groups to determine serum cholesterol, serum glucose and serum insulin.

Biochemical analysis

Serum was obtained by high speed centrifugation at (3500 rmp) for 10 min, and stored at 70°C until analysis. The concentrations of cholesterol and serum glucose were measured by enzymatic colorimetric methods with commercial kits (pars Azmone, IRI) on an automatic analyzer (Abbott, model Alcyon 300, USA) and serum insulin level was determined by ELISA method using insulin kit (DRG, international, Inc, USA).

Statistical analysis

A one-way analysis of variance (ANOVA) and then Tukey test were used to characterize the effects of saffron extract statistically by SAS software (version 9.1). The P-values less than 0.05 were considered to be statistically significant.

RESULTS

Serum cholesterol

On the day before the experiment and after 7days of treatment with saffron extract in the test group, no significant difference in serum cholesterol level was found in the test group compared to sham and normal groups ($p > 0.05$) (Table 1). On the 14th day of treatment with saffron extract in test group, the level of cholesterol significantly decreased compared to the sham and normal groups ($p < 0.05$).There was no statistically significant difference in serum cholesterol between the sham and normal groups ($p > 0.05$) (Table 1). No significant difference in serum cholesterol level was found between the 14th day compared to the day before experiment and the 7th day ($p > 0.05$) (Figure 1).

Serum insulin

As shown in (Table 2), the day before experiment and after 7days of treatment with saffron extract in the test

Table 1. Effect of the intraperitoneally daily administration of hydromethanolic extract of *C. Sativus* L. stigma at a dose of 50 mgkg^{-1} on blood cholesterol levels (mg dl^{-1}) in healthy male rats.

Groups	Days		
	0	7	14
(Normal)	64.5 ± 13.616	65.20 ± 8.257	65.10 ± 7.218 [a]
(Test)	62.00 ± 3.559	61.80 ± 8.867	56.00 ± 8.654 [b]
(Sham)	63.60 ± 8.859	62.70 ± 6.881	63.60 ± 8.343 [a]

a and b: The different letters in any column of table, representing the significant differences between various groups ($p < 0.05$) and the same letters (or no letter on above of groups) representing no significant differences between them.

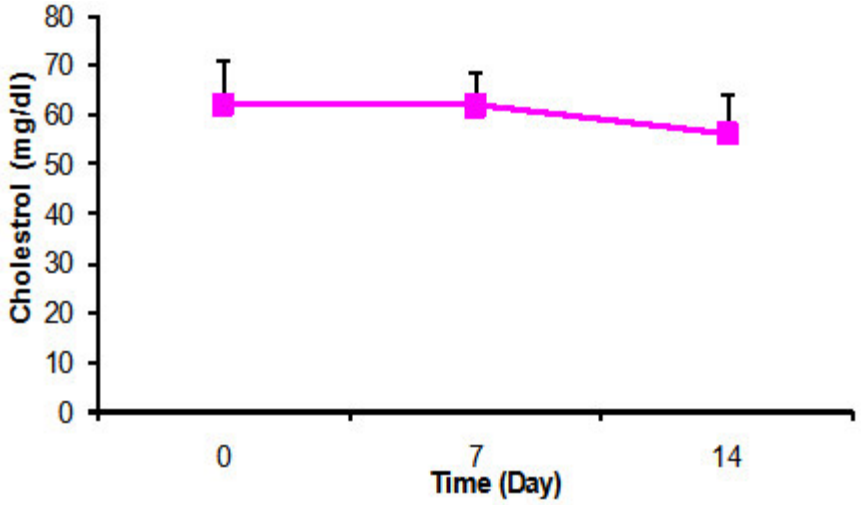

Figure 1. The effects of hydromethanolic extract of saffron treatment period (day) on cholesterol levels in test group of rats. Results denote mean ± SD (n = 10). No significant differences between various time periods (0, 7 and 14 days) was seen based on 5% significance level ($p < 0.05$).

Table 2. Effect of the intraperitoneally daily administration of hydromethanolic extract of *C. Sativus* L. stigma at a dose of 50 mgkg^{-1} on blood insulin levels (μgL^{-1}) in healthy male rats.

Groups	Days		
	0	7	14
(Normal)	1.17 ± 0.389	1.21 ± 0.420	1.35 ± 0.427 [a]
(Test)	1.07 ± 0.305	1.52 ± 0.421	2.03 ± 0.651 [b]
(Sham)	1.33 ± 0.408	1.19 ± 0.228	1.48 ± 0.396 [ab]

a and b: The different letters in any column of table, representing the significant differences between various groups ($p < 0.05$) and the same letters (or no letter on above of groups) representing no significant differences between them.

group, no significant difference in serum insulin level was found compared to the sham and normal groups ($p > 0.05$). There was no significant difference in serum insulin between the sham and normal groups ($p > 0.05$). On the 14th day of treatment with saffron extract, in the test group, the level of insulin significantly increased compared to the sham and normal groups ($p < 0.05$). There was no significant difference in serum insulin between the sham and normal groups ($p > 0.05$). Significant difference was observed in the level of serum insulin between the

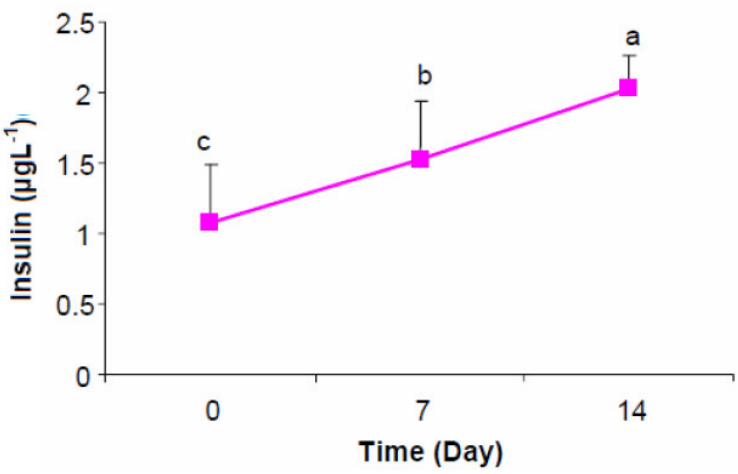

Figure 2. The effects of hydromethanolic extract of saffron treatment period (day) on insulin levels in test group of rats. Results denote mean ± SD (n = 10). The different letters in this figure represent the significant differences between various time periods (0, 7 and 14 days) based on 5% significance level ($p < 0.05$).

Table 3. Effect of the intraperitoneally daily administration of hydromethanolic extract of *C. Sativus* L. stigma at a dose of 50 mgkg^{-1} on blood glucose levels ($mg\ dL^{-1}$) in healthy male rats.

Groups	Days		
	0	7	14
(Normal)	85.3 ± 9.55	92.10 ± 9.73 [a]	93.4 ± 11.4 [a]
(Test)	88.4 ± 12.42	75.4 ± 11.64 [b]	66.20 ± 16.84 [b]
(Sham)	88.6 ± 12.02	91.70 ± 9.99 [a]	92.10 ± 12.04 [a]

a and b: The different letters in any column of table, representing the significant differences between various groups ($p < 0.05$) and the same letters (or no letter on above of groups) representing no significant differences between them.

14th day compared to the day before experiment and the 7th day ($p < 0.05$) (Figure 2).

Serum glucose

On the day before the experiment, there was no signify-cant difference in serum glucose between the test group compared to the sham and normal groups ($p > 0.05$). There was no statistically significant difference between the sham and normal groups ($p > 0.05$). On the 7th day of treatment with saffron extract, the concentration of serum glucose in test group significantly decreased com-pared to the sham and normal groups ($p < 0.05$). After two weeks of treatment with saffron extract in test group, the level of serum glucose significantly decreased compared to sham and normal groups ($p < 0.05$). There was no significant difference between the sham and normal group in glucose level ($p > 0.05$) (Table 3). Significant difference was observed in serum glucose between the 14th day of treatment with saffron extract compared to the day before the experiment in the test group ($p < 0.05$).No significant difference was observed in level of serum glucose between the 14th day and 7th day in the test group ($p > 0.05$) (Figure 3).

DISCUSSION

The results of this study showed that hydromethanolic extract of saffron induces a significant decrease of serum cholesterol levels in saffron group after daily treatment with hydromethanolic saffron extract for 2 weeks, but no changes were observed in the sham and normal groups. Previous studies have shown that the oral administration of crocin decreased serum total cholesterol in rats with diet–induced hyperlipidemia (Du and Qian, 2004; Asai et al., 2005). Their results showed that crocin could not be

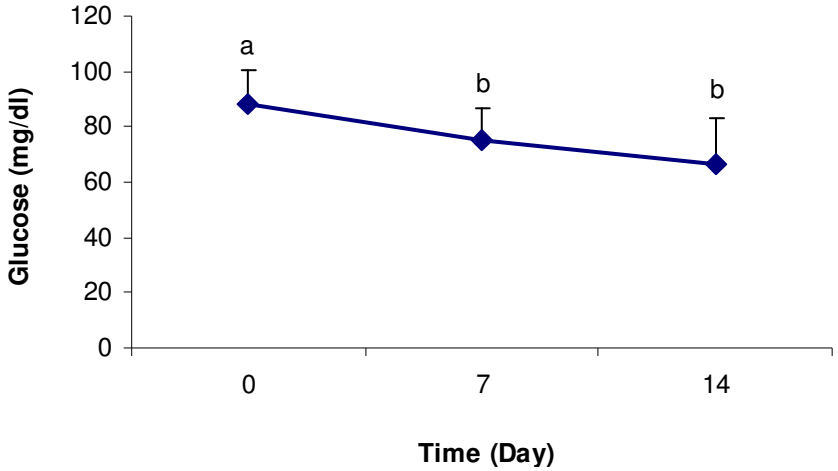

Figure 3. The effects of hydromethanolic extract of saffron treatment period (day) on glucose levels in test group of rats. Results denote mean ± SD (n = 10). The different letters in this figure represent the significant differences between various time periods (0, 7 and 14 days) based on 5% significance level ($p < 0.05$).

absorbed by oral administration. In the elucidation of the hypolipidemic mechanism of crocin, Sheng et al. (2006) indicated that crocin could selectively inhibit the activity of pancreatic lipase as a competitive inhibitor. Zheng et al. (2006) suggested that crocetin prevented atherosclerosis in hyperlipidemic rabbits.

In a recent study, crocetin could reduce the concentration of serum total cholesterol, triglyceride, malondialdehyde and inhibit the descending of nitric oxide in hyperlipidamic-diet quails (He et al., 2006). On the other hand, Radomski et al. (1990) indicated that nitric oxide deficiency could promote arteriosclerosis by facilitating cell migration and proliferation within blood vessel walls. Based on the recent investigation and our results, saffron extract has hypolipidemic effect on rats with oral administration and intraperitoneally injection. In agreement with previous studies, several mechanisms for the hypolipidemic effects of saffron extract and its constituents have been proposed: (1) Inhibitory effects on the levels of malondialdehyde, oxygen free radical and intracellular ca^{2+} concentration in endothelial cell and activiating superoxide dismutase (Xiang et al 2006). (2) Inhibitory effect on pancreatic lipase. It may act by reducing the absorption of fat and cholesterol through inhibiting pancreatic lipase activity (Sheng et al 2006). (3) It was reported that the mechanism by which crocetin reduces the effects of experimental atherosclerosis and increases oxygen diffusivity must reflect strong plasma albumin binding (Miller et al 1982). Regarding the effects of saffron extract on serum glucose, insulin resistance and insulin secretion from pancreatic B-cells, and based on this study, for the first time in healthy rats, a significant reduction was observed in serum glucose after 7 days daily treatment with saffron extract in saffron group compared to the sham and normal groups. Nevertheless, there was no significant difference in serum glucose between the sham and normal groups.

Our findings also showed that hydromethanolic extract of saffron induces significant increase of serum insulin levels in test group after 2 weeks of treatment compared to the normal and sham groups. Based on previous studies, we found that adipocyte-derived factors including free fatty acids (FFA), adiponectin, tumour necrosis factor-α (TNF-α) and leptin are responsible for insulin resistance (Borden, 1997). Recently, Xi et al. (2005) indicated that male wistar rats treated with subcutaneous dexamethasone for 6 weeks exhibited reduced insulin sensitivity and serum insulin, FFA, TG, and TNF-α levels were significantly increased. However, Treatment with crocetin significantly decreased all the described effects of dexamethasone. Interestingly, Mohajeri et al. (2009) indicated ethanolic extract of saffron significantly increased serum insulin and decreased blood glucose levels in diabetic rats, while our results showed that saffron extract significantly reduced blood glucose and increased serum insulin levels in healthy male rats. The hypoglycemic effect of saffron extract seems to exert by mechanisms such as insulin resistance reducing (Xi et al 2007), stimulating of glucose uptake by peripheral tissues (Yang et al., 2003), inhibition of intestinal glucose absorption (Youn et al., 2004) and it seems that hypoglycemic action of other medicinal plants and saffron at least partly is due to antioxidant properties of these plants (Al-Azzawie et al., 2006).

In conclusion, the results obtained in the present investigation suggest that the saffron extract and its active constituent significantly decreased serum glucose, serum cholesterol and increased insulin levels in healthy male rats. However, further extensive work is needed to confirm this data and evaluate these probable mechanisms.

Therefore, saffron may be regarded as a useful therapy for diabetes mellitus and hyperlipidemia.

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