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Full Length Research Paper

The effects of hydromethanolic extract of saffron (*Crocus sativus*) on some biochemical parameters of serum blood in constant darkness and light conditions in healthy male rats

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The purpose of the present investigation was to determine the effects of hydromethanolic extract of saffron on changes of serum cholesterol, glucose and insulin levels in constant darkness and light conditions in healthy male rats. In this study, healthy male rats (n = 30) were divided into 3 groups of tens and kept at various light/dark conditions: Control + saffron 12:12 light/dark (LDS); constant darkness + saffron (DDS) and constant light + saffron (LLS) groups for 2 weeks. All the three groups received 50 mg/kg saffron extract through intraperitoneal injection for 2 weeks. Daily injection was repeated at 10 am. Blood samples were obtained from retro-orbital sinus before administration and on the 7 and 14th days of administration. The serum cholesterol and glucose levels were measured by the enzymatic method and insulin levels were measured using insulin kit by enzyme-linked immunosorbent assay (ELISA) method. The results showed that the serum cholesterol levels of all three groups decreased after 2 weeks of experiment and the most reduction was seen in DDS group. Comparison of serum insulin in all groups did not show any significant changes, but serum glucose level in LLS group significantly increased compared with the two other groups ($P<0.05$). In contrast, the levels of serum glucose significantly decreased in DDS and LDS groups compared to LLS group ($P<0.05$). In conclusion, these results clearly indicated that saffron extract and constant darkness have hypoglycemic and hypolipidemic effects, but constant light condition showed hyperglycemic effect on serum blood in healthy male rats.

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

INTRODUCTION

High concentrations of blood cholesterol are associated with development of illnesses like cardiovascular (Paniangvait, 1995). Medicinal plants are frequently considered to be less toxic and free from side effects than the synthetic ones (Huang et al., 2005; Loew and Kaszkin, 2002). Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies (Shukla et al., 2000; Al-Azzawie and Alhamdani, 2006). *Crocus sativus* L. commonly known as saffron is a perennial stemless herb of the Iridaceae family and widely cultivated in Iran and other countries such as India, Azerbaijan and Greece. The main active constituents of this plant are safranal,

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picrocrocin, crocetin and its glycoside, crocin (Rio et al., 1996). Saffron consists of dried stigmas and top of styles of *C. sativus* (Evans, 2000). In modern pharmacological studies, saffron, or its active constituents has demonstrated antidepressant (Hosseinzadeh et al., 2004), anti -inflammatory (Hosseinzadeh and Younesi, 2002), radical scavenger effects as well as learning and memory improving properties (Zhang et al., 1994; Abe et al., 1999), antioxidant activity (Assimopoulou et al., 2005), insulin resistance reducing (Xi et al., 2005), hypolipedemic (Sheng et al., 2006), antihyperglycemic and pancrease-protective (Mohajeri et al., 2009) effects. Therefore, little is known about effects of saffron on cholesterol, insulin and glucose levels in animal and human. Recently, our investigation has demonstrated that hydromethanolic extract of saffron significantly decreased serum glucose and cholesterol levels after 2 weeks of administration in healthy male rats. Also, our results showed that saffron extract could increase their insulin secretion from pancreatic B-cells (Arasteh et al., 2010a). On the other hand, it is well known that most behavioral and physiological function display daily temporal variations synchronous with the ambient light-dark cycle and these variation are driven by a circadian clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus (Challet et al., 2004). There is evidence of relationship between the circadian clocks, pineal gland and physiological regulation of carbohydrate and lipid metabolism. In this regard, in pinealectomized rats, the decrease of tolerance to glucose, the increase in the level of total lipids, free fatty acids, disturbances in the ratio of free and bounded insulin were observed (Ostroumova and Vasilieva, 1976). In addition, melatonin, an endocrine product of the pineal gland is formed predominantly during night-time (Wurtman et al., 1964). Melatonin plays a key role in a variety of important physiological functions including: regulation of circadian rhythms and cardiovascular (Karasek and Winczyk, 2006).

Our previous work demonstrated that constant darkness significantly decreased serum glucose, insulin and cholesterol levels after 2 weeks of experiment in healthy male rats. However, constant light significantly increased serum glucose level without having any significant effect on serum cholesterol and insulin levels (Arasteh et al., 2010b). Therefore, we investigated the effects of hydromethanolic extract of saffron on change of serum cholesterol, insulin and glucose levels in constant darkness and light conditions in healthy male rats.

MATERIALS AND METHODS

Plant material and preparation of the plant 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, 50 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 (n = 30) weighing 200 to 250 g were obtained from the Pasteur institute of Iran. Animals were housed individually under standard laboratory conditions in a 12/12 h light/dark cycle and at a temperature of 21 to 23°C. Rats had free access to food and water. The rats were maintained under these housing conditions for 15 days before the experiments.

Experimental design

Animals were randomized and divided into three groups of ten as follows: Group 1 - control + saffron (LDS) -12/12 h light/dark cycle: Group 2 – darkness + saffron (DDS) -24 h darkness condition and Group 3 – light + saffron (LLS) – 24 h light on (100 lux) condition. All rats were kept in standard cages and were fed ad libitum standard laboratory meal and tap water (Challet et al., 2004). On the day before the experiment, blood samples were collected from retro-orbital sinus from three groups. After this, the DDS group was transferred to constant darkness condition for 2 weeks. The LLS rats were exposed to a white light pulse (100 lux during 2 weeks). LDS rats were maintained under 12/12 h light/dark condition for 2 weeks of the experimental period. Saffron extract (50 mg/kg) was injected into the three groups intraperitoneally and during a period of two weeks, daily injection of saffron extract to three groups was repeated at 10 am. On the 7 and 14th day as the day before treatment, blood samples were collected separately from the three groups to determine serum cholesterol, glucose and insulin levels.

Biochemical assays

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 enzyme-linked immunosorbent assay (ELSA) method using insulin kit (DRG, international, Inc, USA).

Statistical analysis

One-way analysis of variance (ANOVA) and Turkey tests were used to characterize the effects of saffron extract in darkness and light conditions using the statistical analysis system (SAS) software (version 9.1). P-values less than 0.05 ($P<0.05$) were considered to be statistically significant.

RESULTS

Effect on serum cholesterol levels

Comparison of results obtained from three groups revealed that the day before experiment, there was no

Table 1. Effects of the intraperitoneally daily administration of hydromethanolic extract of *C. Sativus* L. stigma at a dose of 50 mg/kg in constant darkness and light conditions on serum cholesterol levels (mg dl^{-1}) in healthy male rats.

Groups	Days		
	0	7	14
Control + saffron (LDS)	62.00±3.56	61.8±8.86	56.00±8.65
Darkness + saffron (DDS)	62.40±14.14; p>0.05	54.4±7.19; P<0.05	49.70±8.35; p>0.05
Control + saffron (LDS)	62.00±3.56	61.8±8.86	56.00±8.65
Light + saffron (LLS)	73.50±10.66; p>0.05	66.00±7.70; p>0.05	55.40±6.27; p>0.05
Darkness + saffron (DDS)	62.40±14.14	54.4±7.19	49.70±8.35
Light + saffron (LLS)	73.50±10.66; p>0.05	66.00±7.70; P<0.05	55.40±6.27; p>0.05

Values are given as mean ± SD for 10 rats in each group. Experimental groups were compared with each other located in any cell.

Table 2. Effects of the intraperitoneally daily administration of hydromethanolic extract of *C. Sativus* L. stigma at a dose of 50 mg/kg in constant darkness and light conditions on serum insulin levels (µg L^{-1}) in healthy male rats.

Groups	Days		
	0	7	14
Control + saffron (LDS)	1.07±0.305	1.52±0.421	2.03±0.652
Darkness + saffron (DDS)	1.12±0.367; p>0.05	1.18±0.355; p>0.05	1.66±0.818; p>0.05
Control + saffron (LDS)	1.07±0.305	1.52±0.421	2.03±0.652
Light + saffron (LLS)	1.01±0.292; p>0.05	1.14±0.377; p>0.05	1.30±0.605; p>0.05
Darkness + saffron (DDS)	1.12±0.367	1.18±0.355	1.66±0.818
Light + saffron) (LLS)	1.01±0.292; p>0.05	1.14±0.377; p>0.05	1.30±0.605; p>0.05

Values are given as mean ± SD for 10 rats in each group. Experimental groups were compared with each other located in any cell.

significant difference in the serum cholesterol levels among the three groups of rats (P>0.05). On the 7th day of experiment we observed the decrease of serum cholesterol levels in the three groups. Interestingly, there was significant difference in serum cholesterol levels between the DDS and LDS groups and between DDS and LLS group (P<0.05). There was no significant difference in serum cholesterol levels between the LLS and LDS groups (P>0.05). Also, our results showed that on 14th day of experiment, the level of cholesterol in the three groups decreased. However, there was no significant difference in serum cholesterol levels among the three groups of rats (P>0.05). The results are shown in Table 1.

Effects on insulin and glucose levels

Our data showed that on the previous and 7th day of experiment, there was no significant difference in serum insulin among the three groups of rats (P>0.05). After 14 days of experiment we found that the level of serum insulin increased in LDS group compared to DDS and LLS groups. However, the level of serum insulin in LDS group had no significant changes compared to DDS and LLS groups (P>0.05) (Table 2). As shown in Table 3, on the day before experiment, there was no significant difference in serum glucose among the three groups (P>0.05). After 7 days of experiment, our data showed that the serum glucose levels deceased in LDS and DDS groups compared to baseline levels. We observed that the serum glucose level increased in LLS group. After 7 days of experiment, we found significant difference in serum glucose level between LLS and LDS groups (P<0.05). Our findings showed that decrease of the levels of serum glucose continued in both LDS and DDS groups after 2 weeks of experiment. But the level of serum glucose increased in LLS groups after 14 days of experiment. There was significant difference in serum glucose level between LDS and LLS groups after 14 days of experiment (P<0.05). Also, there was significant difference in serum glucose level between DDS and LLS groups (P<0.05). No significant difference in serum glucose level was found between LDS and DDS groups after 14 days of experiment (P>0.05) (Table 3).

DISCUSSION

Results obtained from this study showed that saffron extract after 7 days of experiment decreased the serum cholesterol levels in constant darkness and light

Table 3. Effects of the intraperitoneally daily administration of hydromethanolic extract of *C. Sativus* L. stigma at a dose of 50 mg/kg in constant darkness and light conditions on serum glucose levels (mg dl^{-1}) in healthy male rats.

Groups	Days		
	0	7	14
Control + saffron (LDS)	88.40±12.42	74.4±11.64	66.20±16.84
Darkness + saffron (DDS)	84.90±6.54; p>0.05	79.30±9.36; p>0.05	70.10±15.62; p>0.05
Control + saffron (LDS)	88.40±12.42	74.4±11.64	66.20±16.84
Light + saffron (LLS)	79.90±14.65; p>0.05	87.70±7.74; P<0.05	94.40±11.65; P<0.05
Darkness + saffron DDS)	84.90±6.54	79.30±9.36	70.10±15.62
Light + saffron (LLS)	79.90±14.65; p>0.05	87.70±7.74; P<0.05	94.40±11.65; P<0.05

Values are given as mean ± SD for 10 rats in each group. Experimental groups were compared with each other located in any cell.

conditions. Reduction of serum cholesterol levels continued after 7 days of experiment and maximum reduction occurred on 14th day of experiment in darkness condition in serum blood rats. However, there was no significant difference in the serum cholesterol in DDS group compared to LLS and LDS groups (Table 1). In this regard, our previous studies have demonstrated that 'constant darkness' reduced serum cholesterol after 2 weeks of experiment in healthy male rats (Arasteh et al., 2010b). Also, another of our previous findings have shown saffron extract significantly decreased the level of cholesterol serum in healthy male rats after 14 days of experiment in a 12/12 h light/dark cycle condition (Arasteh et al., 2010a). It seems that the decrease of cholesterol level in darkness condition may be due to melatonin secretion in darkness condition and effects on lipid parameters such as inhibition of low-density lipoprotein (LDL) receptor activity and cholesterol synthesis in human mononuclear leucocytes (Muller-Wieland et al., 1994), decrease of plasma and liver cholesterol levels in hypercholesterolemic mice (Sener et al., 2004). Regarding the effect of circadian clock on cholesterol levels, it has been demonstrated that in mice mutation in circadian clock gene developed metabolic syndrome (Turek et al., 2005). On the other hand, based on recent findings and our data, several mechanisms for the hypolipidemic effects of saffron extract and its constituents have been proposed: inhibitory effect on pancreatic lipase (Sheng et al., 2006) and inhibitory effects on the levels of malondialdehyde, oxygen free radical and intracellular ca^{2+} concentration in endothelial cell and activating superoxide dismutase (Xiang et al., 2006). The present study provides evidence that saffron extract could alter the level of serum glucose in the three groups, but did not change the serum insulin. Our findings showed that there was no significant difference in serum insulin among in the three groups of experiment, although we observed serum insulin increased in LDS groups compared to other groups (Table 2). However, our previous works have demonstrated that after 2 weeks of experiment saffron extract significantly increased the level of serum insulin in healthy male rats (Arasteh et al., 2010a). Also our data in another study showed that after 14 days of experiment in dark condition, the serum insulin significantly decreased in healthy male rats (Arasteh et al., 2010b). Therefore, it seems that effects of constant darkness to be more effective on the level of serum insulin changes and saffron extract was affected by darkness, therefore serum insulin could not alter. This study has demonstrated that saffron extract in constant darkness decreased the serum glucose levels and significantly increased the serum glucose levels in constant light condition in healthy male rats (Table 3). On the other hand, our previous findings have shown that saffron extract significantly decreased the level of serum glucose after 7 days of experiment in healthy male rats.

The hypoglycemic effect of saffron extract seems to exert by mechanisms such as insulin resistance reducing (Xi et al., 2007), stimulation of glucose uptake by peripheral tissues (Yang et al., 2003) inhibition of intestinal glucose absorption (Youn et al., 2004). Also, we showed in another study that constant darkness decreased and constant light increased the level of serum glucose after 7 days of experiment in healthy male rats (Arasteh et al., 2010b). There are a number of documents which strongly support the importance of melatonin in the regulation of circadian function and serum glucose and insulin levels in constant darkness condition (Mazpa et al., 2000; La Fleur et al., 2001; Sankaran and Subramanian, 2006). Despite the similarity of the constant darkness and saffron extract on decrease of serum cholesterol and glucose levels, it cannot be determined whether similar mechanisms are involved in both situations. The findings of the present study and our previous studies are agreement with findings which suggest that constant light increased the level of blood glucose in rats (Challet et al., 2004). Consequently, the main findings of this study was that saffron extract activity was not affected by darkness condition on change of serum cholesterol and glucose levels, but saffron extract activity was affected by darkness on changes of serum insulin and change of serum glucose in constant light conditions. With regard to the present study, our observations led to the hypothesis that saffron extract

could alter the levels of serum cholesterol and glucose levels in constant darkness condition, but did not alter the levels of serum insulin in rats. In addition, saffron extract did not alter the level of serum insulin in light condition, but the level of serum glucose increased in constant light condition. The obtained results suggest that saffron extract and constant darkness have hypoglycemic and hypolipidemic effects, but constant light could show hyperglycemic effect on serum blood in healthy male rats.

Abbreviations: SCN, Suprachiasmatic nucleus; **LDS,** control + saffron group; **LLS,** constant light + saffron group; **DDS,** constant darkness + saffron group; **ELISA,** enzyme-linked immunosorbent assay.

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