

The effects of sour orange (*Citrus aurantium*) extract on sex hormones in diabetic male rats (Wistar)

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Abstract: Diabetes mellitus is a metabolic abnormality which can be one of the reasons of death. According to the importance of diabetic diseases on the one hand, and the effect of sour orange essence on sexual hormones on the other hand, present study was conducted. For this purpose, 40 male rats (wistar), were divided to 4 groups including control group (without treatment), sham (diabetic with streptozotocin) and two diabetic treatment groups that received respectively (100 and 200) mg/kg/b.wt of sour orange essence. After treatment period (56 days), blood samples were taken from all groups and their serum were separated for determining LH, FSH and Testosterone by LISA method. Then testis was dissected, fixed in formalin 10% and sections were prepared by tissue processing for histological study. Data were analyzed by SPSS and ANOVA and the groups were compared by Duncan test in significantly level ($P < 0.05$). Obtained results showed significant reduction in the mean of sermic level of LH and FSH in sham and all treatment groups compared to control group. These hormones in both two treatment groups increased significantly compared to sham group. Also mean of sermic level of testosterone in both treatment groups decreased compared to control group. Also significant reduction in mean number of Leydig cells in all diabetic groups compared to control group was observed and were regenerated in treatment groups with this extract. According to the results of this research, sour orange fruit essence would effect on male sexual hormones in diabetic specimen and it seems that this effect is dose- dependent. So this extract can be considered to reduce the side effects of diabetes on sexual hormones.

Key words: Diabetes; Hormone; Leydig cell; Orange

1. Introduction

Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced. When the body doesn't make enough insulin or is not able to use insulin effectively or both, metabolism disorders is happen (Harrison et al., 2001). So in this type of diabetes, skeletal muscle, liver and adipose tissue will resistance to insulin which can lead to decreased glucose uptake, increased hepatic glucose and lipid (Memisogullari et al., 2003). This disease due to factors as genetic, environmental stimuli, behavior and habits (Harrison et al., 2001).

Previous research showed that diabetes mellitus effects on physiology and anatomy of male reproductive system as decline in sex drive (Soudamani et al., 2001), degeneration of accessory glands (Soudamani et al., 2005), effecting on spermatogenesis (Balasubramanian et al., 1991) and reduction of testosterone production. Prevalence of diabetes break is about 5- 6% In Iran and about 4000000 of people are diabetic (Azizi et al., 2001). Testosterone is the principal male sex hormone and an anabolic steroid with 19 carbons which is derived from cholesterol in Leydig cells and also small

amounts of it is secreted by the adrenal glands (Ganong et al., 2001).

According to the effects of testosterone on function of sex organs, the concentration of this hormone is ideal marker for testis performance (Ganong et al., 2001). Due to successful effectiveness of synthetic drugs, consumption of these drugs have increased in Iran, but production problems, the high cost and side effects were caused to attention to herbal drugs consumption for treatment by most people (Scartezzini and Speroni, 2000). Although many people believe that these products are always safe for them. This is not necessarily true. Herbal medicines do not have to go through the testing that drugs do (Andreoli et al., 2000). Although herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited or improperly used. Some herbs, such as comfrey and ephedra, can cause serious harm. Some herbs can interact with prescription or over-the-counter medicines (Sharanabasappa and Saraswati, 2001; Sharanabasappa et al., 2002).

Herbs have long been used by for preventing and also treating diabetes humans since many years ago, but the effects of many of these haven't been studied. Sour orange (*Citrus aurantium*) belong to *Rutaceae* family (Monsef-esfahani et al., 2004; Stöhs and Preuss, 2011) with single or multiple flowers and

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buds, which has known medicinal properties (. The flowers, bark, fruit and juice of it have medicinal properties (Mahmoodi et al., 2011). The fruit and juice of it is used in treatment of rash or flowers are used in curing nervous disorders such as Hysteria, fit and nerve weakness. Also it is known as sedative-hypnotics, regulator heart palpitations and increase appetite (Tribble et al., 1999).

Sour orange peel composed of flavonoids, Vitamin C, Carotene and Pectin. Flavonoids are aromatic secondary plant metabolites, which have been recognized as important due to their physiological (Harrison et al, 2001) and pharmacological role and their health benefits. Flavonoids exhibit also antiviral, antimicrobial (Mahmoodi et al, 2011). and anti-inflammatory activities (Monsef-esfahani et al, 2004). The compounds of plant leaves, flower and peel are significantly different. 50% of the concentration of lineally acetate is in the leaf essence whereas 35% of flower essence is linalool acetate (Goli et al, 2010).

2. Material and methods

Sour orange fruit were cleaned, dried completely and then grinded. 100 gram of powder of it mixed with 1000 ml of ethanol 50% and extract was prepared. In this study, 40 adult male Wistar rats weighing approximately 170-200- g were selected, preserved in standard conditions (2 ± 22 °C and 12 height and 12 h dark cycle) for a weeks and were divided into 4 groups include control group, sham and 2 diabetic groups (treatment groups). The rats were injected interaperitoneal solution of Streptozotocin in NaCl (0.9%) and 100 ml sodium citrate (pH = 4.5).

Blood sugar was determined in specimens before Streptozotocin injection and seven days after injection. Control group (without treatment), Sham group (diabetic and received streptozotocin) and two diabetic treatment groups that received respectively (100 and 200) mg/kg/b.wt of sour orange essence. After treatment period (56 days), blood samples were taken from all groups and their serum were separated for determining LH, FSH and Testosterone by ELISA method. Data were analyzed by software SPSS version 18 and ANOVA test then the groups were compared by Duncan test in significantly level (P<0.05).

3. Results

Obtained results of this research showed that significant reduction in the mean of sermic level of LH and FSH in sham (Fig. 2, 3) and all treatment groups compared to control group (p≤0.05). These hormones in both two treatment groups increased significantly compared to sham group. Also mean of sermic level of testosterone in both treatment groups decreased (Fig. 1), sham group (diabetic with Streptozotocin) and two diabetic treatment groups

that received respectively (100 and 200) mg/kg/b.wt of sour orange extract.

According to the results of histological micrographs and counting cells, meaningful reduction in mean number of Leydig cells in all diabetic groups compared to control group was observed but in treatment groups with sour orange extract in high dose was regenerated (Table 1).

4. Discussion

Diabetes will lead to disorder in Spermatogenesis and reduction in testosterone level (Ravanshad et al, 2009).

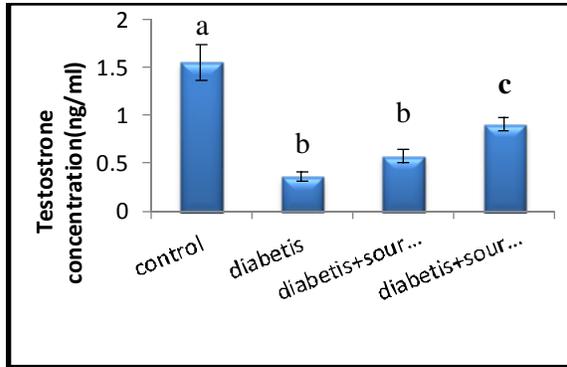


Fig. 1: Seromic level of Testosterone concentration in rats

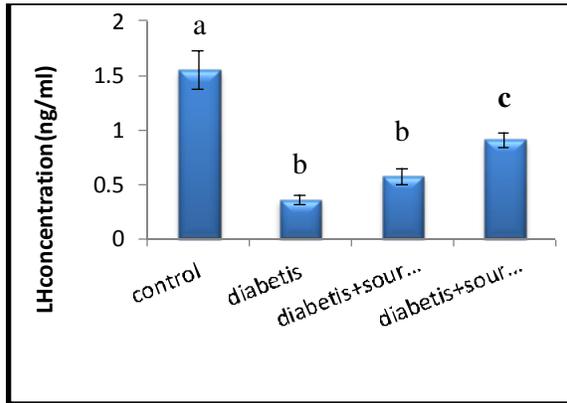


Fig. 2: Seromic level of LH concentration in rats

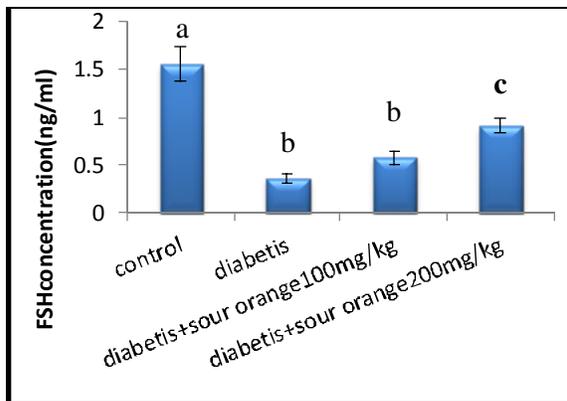


Fig. 3: Seromic level of FSH concentration in rats

Table 1: Density of Leydig cells in rats

Leydig cell ($\bar{X} \pm SEM$)	N	Treatment Group
4.77a±26.72	10	Control
1.35 3b±0.28	10	Diabetic
1.03 b±4.1	10	Diabetes +Sour Orange 100mg/kg
4.77 b±9.02	10	Diabetes +Sour Orange 200mg/kg

According to "Tradition medicine of Iran" text some herbs which can be effective in diabetes treatment and also can be useful in stimulating and improving sexuality are as Fenugreek, Ginger, Nettle, Raspberry, banana, cabbage, red and green pepper, Cucurbit seed, Succor and licorice. Previous finding showed that they have plants anti-oxidant materials which are so much effective in treating infertility in diabetic men (Ganong et al., 2001; Khaki et al., 2010).

Studies showed that daily uptake of fresh sour orange juice for 4 weeks in diabetic patients with dyslipidemia would lead to a meaningful reduction in FBS and also a meaningful increase in vitamin C (Tribble et al., 1999). Using aqueous essence of onion plant also will lead to reduction in symptoms resulted in by diabetes effect on male reproductive system (Hosseini et al., 2010). The level of antioxidant activity in these plants varies greatly, and some of the compounds as phenolic and flavonoids in the plant extracts provided a substantial antioxidant activity (Ghasemi et al., 2014). Experimental results indicate that flavonoids show a strong antioxidant and radical scavenging activity and appear to be associated with reduced risk for certain chronic diseases (Nikroosh et al., 2007).

In this study has been observed that sour orange essence in both doses (100 and 200 mg/kg) have been increased the concentration of LH and FSH hormone compared to sham group. Probably used extract as antioxidant so prevent of oxidative stress (Goli et al., 2010). Metabolism of many compounds by cells as genital cell or leydig cell causes an increase in the levels of the free radicals that can react with oxygen causing an increase in ROS (Varnet et al., 2004).

The observation in this study indicate that high dose of the used extract was more effective, may be the effect of it is dependent on dose so, finding effective dose in different treatment is very necessary. Reduction of testosterone concentration due to reduction in density of Leydig cells and this is responsible for tissue changes in testicular tissue. The effect of diabetes on some organs such as testis is confirmed but usage some compound with antioxidant properties as Phenolic and flavonoids are effective in reduction of free radicals. These compounds are derived from sour orange essence (Sonmez et al., 2007), have been used as antioxidant agents. The results of this study and significant decrease in all hormones level, by-product of lipid peroxidation confirmed (Henkel et al., 2011).

This result was observed in study of barberry juice due to possessing anti-oxidant properties which lead to testosterone increase in diabetic rats treated with essence (Sonmez et al., 2007). Vitamins

E and C and beta-carotene with having anti-oxidant properties are so effective in decreasing risk of many diseases including cardiovascular and its presence in sour orange essence can consider as a anti-oxidant property which prevent forming ROS and lipid peroxidation (Huang et al., 2011).

5. Conclusion

In conclusion, diabetes by disturbances in testicular somatic cell functions (Leydig cells) and germ cell cause to change histophysiological events of spermatogenesis. According to obtained results, sour orange essence have very immense effects against symptoms resulted by diabetes on sexual hormone. Probably its effect is dependent on dose and consumption dosage is so much important in creating the effect so, therefore usage of it is suggested for the diabetic patients.

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