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Effect of Saffron on Histomorphometric Changes of Testicular Tissue in Rat

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Abstract: Problem statement: Saffron is widely used as a food flavor and has well known medicinal effects. This drug has an effect on steroid and sexual hormones. The aim of this study was investigation of Saffron orally administration on histomorphometric change of testicular tissue in rat. In this experimental study, 40 male rats are used (4 groups and 10 rats in each group). **Approach:** Animals were received Saffron (50, 100 and 200 mg kg^{-1}) daily orally administration, during 28 days. As one group was control (normal saline) and three groups was drug consumer. The testis tissue was sampled after passing of above time and section providing, was stained by H and E. **Results:** The histomorphometric changes of testicular tissue which include, seminiferous tubules diameter, seminiferous epithelial thickness, interstitial tissue and testicular capsule thickness were studied. Results showed that seminiferous tubules diameter, seminiferous epithelial thickness had significantly decreased ($p<0.05$) but interstitial tissue thickness had significantly increased ($p<0.05$) compared with control group. Testicular capsule thickness had not significantly changed. **Conclusion:** Our results showed that seminiferous tubules diameter, seminiferous epithelial thickness and interstitial tissue thickness in rat testis were significantly changed after Saffron orally administration. This results probably via the reducing of serum testosterone concentration. Effect of Saffron on spermatogenesis index and unfertilization in human needed to be more investigation.

Key words: Testicular tissue, tubules diameter, seminiferous epithelial, interstitial tissue, tissue thickness, results showed, capsule thickness, testis tissue, testicular capsule

INTRODUCTION

Applying medicamental herbs has been popular from ancient times among people and in recent years a multilateral approach has emerged on using medicines with natural and especially herbal origin (Mohajeri *et al*., 2007; 2008). Medicamental herbs like industrial drugs may cause some irretrievable tissue damages through its unwanted side effects. Evaluating side and toxic effects of medicamental herbs by performing experimental tests on animal models will have an effective advantage on identification and recognition of medicines harmful effects on humans. On the other hand, recognizing the damages on different tissues and organs of body following the use of medicamental herbs will provide an appropriate strategy in order to specify the consumption of these drugs. Determining the dosage of medicine is very important too, for overdose of herbal medicines may not only not cure the illness, but also cause some irreparable damages (Mohajeri *et al*., 2008; Smet *et al*., 1997). Saffron (*Crocus sativus*) from iridaceous genus is a stable grass with a height about 30 cm with long and green leaves. Saffron's flowers are purple, well ordered with a long tube that ends to 3 petals and sepals. The interested segment of saffron is its stigma. Saffron is considered as an important herb in medical, cosmetics and hygienic industries (Abdullaev, 1993; Mohajeri *et al*., 2007; 2008). Saffron has a sweet smell and a biting taste which has been in use from long times before as a seasoning and food color (Fatehi *et al*., 2003). Regarding therapeutic characteristics, saffron is beneficial for curing nervous pains, insomnia, paroxysm, asthma, rheumatism, gingivitis, cough, gastric disorders, sleeplessness, uterus chronic hemorrhage, femininity disorders, scarlet fever, influenza and cardiovascular disorders and brain

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damages (Bisset and Wichtl, 2001; Fatehi *et al*., 2003; Gainer and Jones, 1975; Mohajeri *et al*., 2008; Wuthrich *et al*., 1997). In traditional medicine, saffron has been utilized with various applications such as sexual potential stimulant (Abe and Saito, 2000), anti spasm (Bisset and Wichtl, 2001; Hosseinzadeh and Talebzadeh, 2005) anti depression (Moshiri *et al*., 2006), sedative and anti inflammation, anti flatulence, regulating menstruation, increasing factor of body transpiration (Abe and Saito, 2000; Abdullaev and Espinosa-Aguirre, 2004; Abdullaev and Ferenkel, 1992; Akhondzadeh *et al*., 2004; Fatehi *et al*., 2003; Meistrich *et al*., 2003; Hosseinzadeh and Talebzadeh, 2005; Mohajeri *et al*., 2007; 2008; Moshiri *et al*., 2006; Ochiai *et al*., 2007). Also it has been cleared that this grass improves the memory power and has some removal effects on free radicals. It also has anti tumorous properties (Jafarova *et al*., 2002; Abdullaev and Ferenkel, 1992; Escribano *et al*., 1996; Garcia-OImo *et al*., 1999; Nair *et al*., 1995). Saffron's extract effect on declining blood pressure and its loosening influences on smooth muscles on rats and guinea pigs has been examined (Fatehi *et al*., 2003). Researchers have demonstrated that saffron's extract reduces ischemia and blood reestablishment in kidney and skeleton muscles (Hosseinzadeh *et al*., 2009; Hosseinzadeh *et al*., 2005; Hosseinzadeh and Younesi, 2022). Saffron's potential anti arrhythmias role has been reported in curing supraventricular tachycardia arrhythmias. Saffron's extract's anti diabetic effects and its efficiency in reduction of blood's sugar and fat amount is also proven (Bisset and Wichtl, 2001; El Daly, 1998; Gainer and Jones, 1975; Mohajeri *et al*., 2008). The studies have displayed that saffron's extract has anti tumorous (Abdullaev and Espinosa-Aguirre, 2004) and anti mutation (Fernandez, 2004) properties and prevents the nucleic acid synthesis in malignant cells on humans (Abdullaev and Ferenkel, 1992; Mohajeri *et al*., 2008; Nair *et al*., 1995). Saffron's extract has various compounds like α-Krustyn, crocins including the crocin and tricrocin, pykrvkrvsyn and safranal (Alonso *et al*., 1998; Li *et al*., 1999; Lozano *et al*., 2000; Tarantilis *et al*., 1994; 1995). These carotenoids protective effect on variety of cancers and preventing exhaustion of neurons and enhancing the memory have been studied (Ochiai *et al*., 2007; 2004) but there has been insufficient attention to physiologic or side effects of saffron's extract on reproductive system function (Fernandez, 2004). Regarding valuable alkaloids due to the body of eruption and saffron's root in majority of pharmacopoeia, it has been introduced as a very important herbal medicine and its properties have been stated. This herb, enjoying valuable alkaloids like lycopene and crocin (that both have anti tumor functions), has got a special place in pharmaceutics (Abdullaev, 1993; Nair *et al*., 1995). The most significant medical advantage of saffron is in curing prostate cancer. This disease emerges due to hormonal changes such as decreased andrstron or increased serum estrogen, estradiol and androgen amounts. Alkaloid lycopene through reducing the androgens leads in epithelial cells atrophy and prevents the androgen effect on tissues; this way it can cure the prostate cancer (Nair *et al*., 1995). Considering contradictory reports about toxicity of saffron, there has been confusion on applying it as a herbal medicine. Some reports suggest that saffron is fully nontoxic herb (Abdullaev *et al*., 2003; Abdullaev and Espinosa-Aguirre, 2004). Contrasting this it has been specified that krustyn has some teratogenic impacts (Martin *et al*., 2002). Regarding toxic effects of saffron, its extract has been recognized fairly toxic (Loomis and Hayes, 1996). The undeniable fact is that consuming excess amounts of saffron would be highly toxic, but it has been reported that even using law amounts of it may cause poisoning or signs like vomiting, hematuria, diarrhea and jaundice (Bisset and Wichtl, 2001). It has been also reported that high dosages of saffron result in abortion of fetus (Bisset and Wichtl, 2001; Mohajeri *et al*., 2007; 2008). Testis's as a reproducing gland pair are placed in scrotum. Its endocrine part is recognized through excretion of hormones like testosterone, estrogen, inhibin by lydic and sertoli cells. The exocrine part contains seminiferous tubules that produce and free spermatosoid. Every testicular lobe has one to four seminiferous tubules and it's observed two types of cells in epithelium of these seminiferous tubules; sertoli cells and spermatosoid cells. These cells are divided frequently in basal membrane and transform into spermatosoid after distinctive steps. Gametes during the development replace in cavities on lateral and top walls of sertoli cells or get surrounded completely by these cells. Sexual cells discriminate gradually from pipes base toward the middle hole and their reproduction causes the cells to be expelled into inner cavity of the pipe. During spermatogenesis process, spermatogony cell transform into spermatosoid following necessary divisions and changes. During spermatositogenesis stage, the spermatogony cells perform the mitosis division frequently and become another cells similar to them and eventually turn into primary spermatocyte cells. The primary spermatocytes are the largest sexual cells in seminiferous tubules and occupy the middle part of epithelium; their characteristic sign is the existence of some entangled chromosomes in their nucleus. In first meiosis division, smaller cells called

secondary spermatocytes emerge. By the end of the second meiosis division, the secondary spermatocyte turn into two smaller cells namely spermatide. Nucleuses of spermatides contain some dark chromatin areas. Following a complicated procedure called spermeiogenesis spermatides turn into spermatosoid. Various factors affect spermatogenesis among which it can be mentioned hormonal and physical factors. Some of these hormones are testosterone, Luteinizing Hormone (LH), Follicle Stimulant Hormone (FSH), estrogens and growth hormone. In addition to mentioned hormones the other hormones of pituitary gland such as prolactine and Thyroid Stimulant Hormone (TSH) have secondary role in supporting the testicle activity (Dellman and Eurell, 1998; Hafez and Hafez, 2000). According to effect of saffron on hormones synthetics specially testosterone (Nair *et al*., 1995) and the role of this hormone on spermatogenesis (Dellman and Eurell, 1998) and by attention to importance of male reproductive system (specially testis) and in addition to so fare any article on effect of saffron on spermatogenesis has not been reported, therefore the aim of present study was to determine the long time orally administration of saffron with different dosages on histomorphometric changes of testicular tissue in rat.

MATERIALS AND METHODS

40 Adult Wistar male albino rats weighing between 250 and 300 g were used for the study. They were kept under standard laboratory conditions and were fed with commercial rat pellets and drinking water ad libitum. The animals were housed in polypropylene cages. The keeping and feeding conditions were identical for all animals. These animals were fed through a commercial plate and their used water was supplied from ordinary water. The rats were kept in 23±2co temperature and under 12 h. light and 12 h. dark conditions. These rats divided into 4 groups, each of them containing 10 rats. These rats received 50, 100 and 200 mg kg^{-1} saffron extract daily for 28 days orally. One group (the control group) received normal saline and the other 3 groups received saffron. The applied saffron in this study was supplied from Sahar Khiz Saffron Company (Mashhad, Iran). Firstly the saffron stigma completely powdered using mechanical mill. Extracting saffron, 100 mg of saffron powder dissolved in 5 L. physiology serum solution. The resulted solution kept in room temperature (25 centigrade) for 2 h. We weight the dry centrifuge in a pipe and spilled it into the pipe and centrifuged that with 5000 rounds min^{-1} rate. Then we took the upper solution completely out and put the pipe along with sediments in fur device for 24 h. to get fully dry. Then we weighed the centrifuge pipe and dried sediments to evaluate the unsolved material's weight; subtracting this value from the primary amount the dissolved material's weight was calculated. After computing the dissolved material's weight, the upper solution volume added so that its concentration reached 10 mg mL^{-1}. After orally administration of saffron with these dosages, a portion of testis tissue from each group was preserved in a 10% formaldehyde solution for Histomorphometric studies. Hemotoxylin and eosin were used for staining and later the microscopic slides of the testis were photographed at a magnification of ×40. For determine the Histomorphometric changes in testis tissue, 4 parameters consist of: Thickness of seminiferous duct, seminiferous duct epithelium, capsule and interstitial tissue, Ofcourse for doing this stages of scaled light microscope 10× (Nikon) were used. Values were represented as mean ± SEM. Data were analyzed by one-way Analysis Of Variance (ANOVA) followed by Dunnett's test using Statistical Package for Social Sciences (SPSS) version 10. $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

It was recognized in histological studies on control group of rats that testicle is surrounded by a condensed connected tissue capsule from outside and there is a simple squamous cells layer around it. There is a connected tissue from weak kind under the epithelium which is immediately extends white curtain thread white connected tissue. It can be observed in the middle and depth of white curtain some blood veins crosses especially filled and bloody vessels. Often in areas that connected capsules create connected lamellas, depth in capsules one can observe arteries crosses too. In connected tissue of testicle's capsule, type I collagen fibers, fibrocytes with long and dark nucleuses, fibroblasts with round or ellipse light nucleuses and also smooth muscles scattered in depth of testicle's depth are seen. Inside the testicle's tissue, seminiferous duct encircle the major part of testicle's parenchyma and the cross tissue between the pipes is observed as thin connected lamellas Fig. 1. Histological studies of testicle 28 days after saffron prescription in 50 mL kg^{-1} dosage cleared that the germinal cells number doesn't show significant difference comparing control group. It can be seen sperms collection inside the seminiferous duct Fig. 3. Histological studies of testicle 28 days after saffron prescription in 100 mL kg^{-1} dosage cleared that it results in considerable reduction in germinal cells number especially spermatide cells.

Table 1: Comparison thehistomorphometric changes of testicular tissue in rat after saffron orally administration

Parameter Group	Thickness of seminiferous duct (Micrometer)	Thickness of seminiferous duct epithelium (Micrometer)	Thickness of capsule (Micrometer)	Thickness of interstitial tissue (Micrometer)
Control group	332.66±12.49	86.5±5.5	68.75±6.77	29.25±2.27
Saffron (50 mg kg^{-1})	331.05±14.55	82.24±4.28	67.2±5.45	28.6±3.5
Saffron (100 mg kg^{-1})	346.3±13.66	83.18±5.29	66.84±3.28	24.53±3.66
Saffron (200 mg kg^{-1})	249.12±9.87*	65.4±3.65*	64.53±7.82	38.36±2.21*

*A significance different in comparison with control group ($p<0.05$)

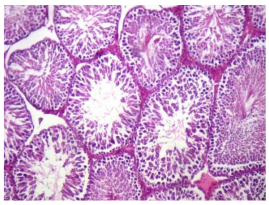

Fig. 1: Photomicrograph of seminiferous tubule in testis of control group rats (H and E×100)

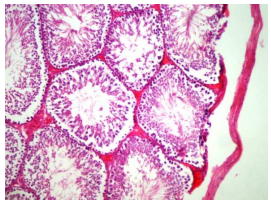

Fig. 2: Photomicrograph of seminiferous tubule in testis of group with 200 mg kg^{-1} saffron administration (H and E×40)

There are sperms inside a few spermatogenesis pipes. Increasing the blood vessels crosses comparing control group is considerable. Histological studies of testicle 28 days after saffron prescription in 200 mL kg^{-1} dosage cleared that the space between pipes (interstitial tissue) enjoys a wide extent. In seminiferous duct the germinal epithelium has less density and in some pipes the spermatogenesis cells series are completely condensed, their number is decreased and the most remaining cells are spermatogony cells. In few numbers of spermatogenesis pipes, germinal epithelium cellular layers and also few numbers of spermatosoid cells with long flagellum, but clearly most of spermatogenesis cells aren't in existence. Sertoli cells show fewer changes. Inside interstitial tissue the blood veins are observed in wide ranges which are seen as arterioles and venues in addition to capillaries. Lydic cells are in natural shapes but in some areas it seems that their assembly is too much Fig. 2 and 3. Also the seminiferous duct diagonal and the density and number of germinal epithelium cells have reduced. Of course still in some pipes there is limited spermatogenesis and some contain spermatosoid cells.

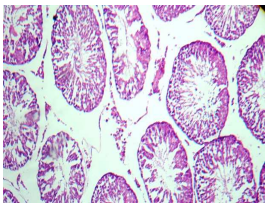

Fig. 3: Photomicrograph of seminiferous tubule in testis of rats after 28 day saffron administration with dosage of 200 mg kg^{-1} (H and E×400)

Interstitial tissue extends widely and often inside the tissue the plasma liquid dissemination is seen as tissue edema in even pink color. Abundant blood vessel crosses in the surface of arteriole and venue and hemorrhage are observed on testicle tissue cross. At some seminiferous duct, whole spermatosoid cells have been removed and there are just limited numbers of spermatogony. Testicle capsule doesn't show significant difference comparing previous Groups Fig. 1-3). From statistical analysis aspect for comparison the thickness of seminiferous duct and seminiferous duct epithelium shows; in dosages of 50 and 100 mg kg^{-1} after saffron administration, thickness of these parts in comparison with control group a significance difference doesn't show but in group with dosage of 200 mg kg^{-1} show a significance reduce in comparing with control group, also significance difference for interstitial tissue thickness between group with dosages of 50 and 100 mg kg^{-1} and control group were observed. In present study thickness of connective capsule significance difference doesn't show. All results of this study in Table 1 have been shown.

CONCLUSION

In present study, saffron's consumption on high dosage caused decreasing the seminiferous duct thickness and germinal cells number among rats. In this survey mostly the spermatide and spermatozoa cells affected, while its consumption on low dosage resulted in insignificant stimulation on testicle tissue and spermatogenesis procedure. The majority changes that

occur on testicle tissue and spermatogenesis procedure following saffron's consumption are probably due to changes in testosterone amount (Nair *et al*., 1995). Saffron is also applied as a traditional anticancer drug on curing advanced prostate cancer due to its prevention effect on testosterone (Nair *et al*., 1995). On a survey performed on mice, saffron consumption with 100 mg kg^{-1} dosage during 20 days resulted in increased FSH, LH and testosterone serum amount. Saffron may reduce hypophyseal-hypothalamus sensitivity to testosterone feedback control on LH secretion. According to results of present study from thickness of seminiferous duct, seminiferous duct epithelium, interstitial connective tissue and connective capsule as histomorphometric index for testicular tissue were used and these indexes are for determine the activity of testicular tissue. Results of this study indicated that changes in these indexes were in testicular tissue of sub treatment rats group with dosage of 200 mg kg^{-1}. Regarding the results of 50 and 100 mg kg^{-1} dosages of saffron, it can be said that through the effect of existed compounds in saffron's stigma as FSH, LH and testosterone hormones stimulant the epithelial cells reproduction in seminiferous duct and increasing the lydic cells activity get provided and leads to insignificant increase on spermatocytes and spermatogenesis amount. Of course saffron's extract can affect directly on spermatogenesis and steroidogenesis processes of lydic cells. According the free radicals theory, imbalance among pro oxidants and anti-oxidants eventually causes oxidative damages on cellular processes and steroidogenesis in lydic cells (Luo *et al*., 2006). Based on Premkumar *et al.* (2003) report, saffron due to its carotenoid compounds results in regulating the peroxidase activity of lipids, anti-oxidants and detoxification. Ochiai *et al*. (2004) have reported that saffron's crocines through indirect increasing of m-RNA enzyme, gamma glotamilsyteienilsyntethaseincrease the restored glutathione amount inside the cell and have a considerable importance in regulating the peroxidase activity of lipids and anti-oxidants. Saffron through enhancing the defensive anti-oxidant system not only reduces the oxidative stresses but also increases the life time and numbers of spermatosoids (Cao *et al*., 2004; Ochiai *et al*., 2004). The conclusions of this research are also confirming saffron's popularity in traditional medicine as an enhancing the sexual power drug (Abdullaev *et al*., 2003). According to these results, saffron's extract can modify reproductive activities in male laboratory mice. It seems that the 100 mg kg^{-1} 48 h. concentration of saffron has the most impact in this context. Also studied the impact of herbal capsule of royal tanetex on LH and FSH hormones amount demonstrating that these herbs cause significant growth in anterior pituitary secretion cells number. These are in accordance with the results suggested that on herbal drug-tanetex fourt-with a similar combination to royal tanetex. Also it has been demonstrated in a survey in India that using royal tanetex capsule increases the mating and ejaculation number and decreases the mating and ejaculation time in wistar mature mice. Also studying colored tissue of anterior pituitary cleared distinct accretion in basophile cells which are responsible for LH and FSH production and significant increase on blood testosterone amount and sperms number. It this study the possible stimulant effects of low dosages of saffron's extract on testicle's tissue and spermatogenesis probably due to increased LH, FSH and testosterone hormones' concentration are examined. Regarding saffron's anti oxidants' effects in steroid hormones biosynthesis it seems that saffron can affect the male sexual hormones concentration. The achieved results on low dosages of saffron in this research also confirm the saffron's fame in traditional medicine as an enhancing drug about sexual power (Abdullaev *et al*., 2003). On the other hand, this study's results revealed that 200 mg kg^{-1} dosage of saffron has had inhibitory and toxic effects on testicle' tissue and spermatogenesis. It has been also recognized that consumption of saffron prevents testosterone synthesis in humans temporarily (Nair *et al*., 1995). Previous studies supported the poisonous impact of saffron too. Researchers have shown that appetite reduction is considered as a side effect following the treatment by saffron (Moshiri *et al*., 2006; Noorbala *et al*., 2005). Mohajeri *et al.* (2008) suggested that significant growth of leukocytes number may be considered as an outcome due to inflammation reactions created in damaged tissues of mice including liver and kidney following the treatment using saffron extract. Saffron's stigma full extract causes normochromic-normocytic anemia among mice. Studies imply that the resulted anemia may be emerged due to bone marrow suppression (Mohajeri *et al*., 2008). Mohajeri *et al.* (2008) also reported that in mice treated by saffron full extract there has been a significant increase in index enzymes of Alannineterasaminas (ALT) and aspartate aminas (AST) which implies damage in liver tissue (Mohajeri *et al*., 2008). Also mice treated by saffron full extract have shown a significant growth in urine serum, uric acid and creatinine levels which indicates a disorder in kidneys' function (Mohajeri *et al*., 2008). A survey by Mohajeri *et al.* (2008) displayed that high amounts of saffron (400 mg) increases serum levels BUN and creatinine in humans (Mohajeri *et al*., 2008).

Histopathological studies indicated that saffron causes considerable damages in liver and kidneys tissues among mice treated by saffron stigma total extract (Mohajeri *et al*., 2008). Degenerative and necrosis changes of hepatocytes in central lobe area is a common phenomenon as this area receives the least oxygen from blood and for this reason is sensitive to hypoxia. Degeneration and necrosis around lobe occurs following the poisonings (Mohajeri *et al*., 2008). According to these results, saffron extract can modify reproductive activities in male rats. However more studies are necessary to demonstrate the mechanisms of saffron impacts. In end can be said; results of present study indicated that high dose administration of saffron causes reduce the thickness of seminiferous duct and seminiferous duct epithelium, however causes increase the thickness of interstitial testicular tissue and reduce the spermatogenesis in rats. These reduce in amount of seminiferous duct and seminiferous duct thickness possibility causes by changes in seral concentration of testosterone. Of course the effect of saffron on spermatogenesis process and human infertility are needed to be more investigated.

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