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**ORIGINAL ARTICLE**

## The effect of Saffron Orally Administration on Spermatogenesis Index in Rat

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### ABSTRACT

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 spermatogenesis index in rat. In this experimental study, 40 male rats are used (4 groups and 10 rats in each group). Animals were received Saffron (50, 100 and 200 mg/kg) 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&E. Then, in point of spermatogenesis indexes which include, tubular differentiation index (TDI), spermatogenesis index (SI), and repopulation index (RI), were studied. According to the results in the 200 mg/kg dose, RI, SI and TDI significantly decreased ( $p < 0.05$ ) compared with control group. Reduction of spermatogenesis and its index in rat testis is the result of Saffron orally administration probably via the reducing of blood testosterone level. Effect of Saffron on spermatogenesis index and unfertilization in human needed to be more investigation.

**Key words:** Saffron, Spermatogenesis Index, Testicular Tissue, Rat.

### 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 [39,42]. 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 [42,14]. Saffron (*Crocus sativus*) from iridaceous genus is a stable grass with a height about 30cm 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 [2,38,39,42]. Saffron has a sweet smell and a biting taste which has been in use from long times before as a seasoning and food color [39,18]. Regarding therapeutic characteristics, saffron is beneficial for curing nervous pains, insomnia,

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paroxysm, asthma, rheumatism, gingivitis, cough, gastric disorders, sleeplessness, uterus chronic hemorrhage, femininity disorders, scarlet fever, influenza and cardiovascular disorders and brain damages [11,18,20,38,39,42,54]. In traditional medicine, saffron has been utilized with various applications such as sexual potential stimulant [1], anti spasm [11,27], anti depression [43], sedative and anti inflammation [38], anti flatulence, regulating menstruation, increasing factor of body transpiration [1,5,6,9,18,27,28,38,39,42,43,46]. 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 [4,6,17,21,44]. Saffron's extract effect on declining blood pressure and its loosening influences on smooth muscles on rats and guinea pigs has been examined [18]. Researches have demonstrated that saffron's extract reduces ischemia and blood reestablishment in kidney and skeleton muscles [25,26]. Saffron's potential anti arrhythmias role has been reported in curing supraventricular tachycardia arrhythmias [30]. Saffron's extract's anti diabetic effects and its efficiency in reduction of blood's sugar and fat amount is also proven [11,16,20,41,42]. The studies have displayed that saffron's extract has anti tumorous [5] and anti mutation [19] properties and prevents the nucleic acid synthesis in malignant cells on humans [6,42,44]. Saffron's extract has various compounds like  $\alpha$ -Krustyn, crocins including the crocin and tricocin, pykrvkrvsyn and safranal [8,10,32,35,38,39,52,53]. These carotenoids protective effect on variety of cancers and preventing exhaustion of neurons and enhancing the memory have been studied [46,51], but there has been insufficient attention to physiologic or side effects of saffron's extract on reproductive system function [19,38,39]. 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 pharmaceuticals [2,44]. 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 [44]. 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 [3,5,7, 48]. Contrasting this it has been specified that krustyn has some teratogenic impacts [36]. Regarding toxic effects of saffron, its extract has been recognized fairly toxic [24,34]. 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 [11]. It has been also reported that high dosages of saffron result in abortion of fetus [11,15, 42]. Testis's as a reproducing gland pair are placed in scrotum. Testis's is considered as a complex gland. 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 spermatozoid. 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 spermatozoid cells. These cells are divided frequently in basal membrane and transform into spermatozoid 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 as adjusted epithelium hold in four to eight layers beside each other and cover the seminiferous tubules density. These 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. The various stages of productive cells distinction in seminiferous tubules are called spermatogenesis. During this process, spermatogony cell transform into spermatozoid following necessary divisions and changes. Spermatogenesis cycle involve three mitosis or spermatositogenesis, meiosis and spermeiogenezia or metamorphose divisions. 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 spermatozoid. 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 [13,23]. According to effect of saffron on hormones synthetics specially testosterone

[38,44] and the role of this hormone on spermatogenesis [13] and by attention to importance of male reproductive system (specially testis) and in addition to so far 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 spermatogenesis index in rat.

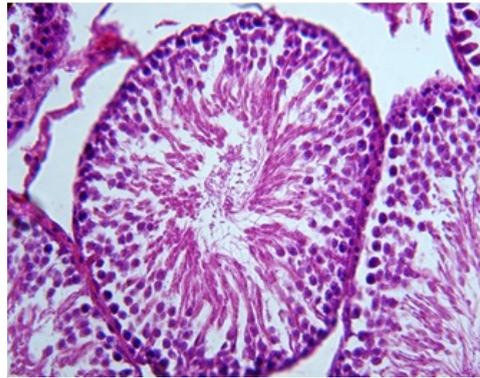
### Material and method

40 Adult Wistar male albino rats weighing between 250 and 300 gr 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  $23\pm 2^{\circ}\text{C}$  temperature and under 12 hours light and 12 hours dark conditions. These rats divided into 4 groups, each of them containing 10 rats. These rats received 50, 100 and 200 mg/kg 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, 100mg of saffron powder dissolved in 5 liters physiology serum solution. The resulted solution kept in room temperature (25 centigrade) for 2 hours. We weight the dry centrifuge in a pipe and spilled it into the pipe and centrifuged that with 5000 rounds per minute rate. Then we took the upper solution completely out and put the pipe along with sediments in fur device for 24 hours 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 10mg/ml [38,39]. After orally administration of saffron with these dosages, a portion of testis tissue from each group was preserved in a 10% formaldehyde solution for histopathological studies. Hemotoxylin and eosin were used for staining and later the microscopic slides of the testis were photographed at a magnification of  $\times 40$ . In present study from 3 parameters consist of TDI, spermeiogenesis index (SI) and repair index (RI) were used. For calculation the TDI from percent of seminiferous ducts that these ducts are consist of interstitial spermatogony, B-type spermatogony, spermatocyte and spermatide were used. In other step for determine the RI from ratio of

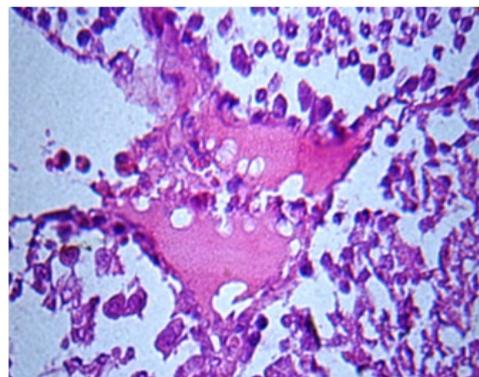
active spermatogony cells to inactive spermatogony cells and also for calculation the SI from ratio of seminiferous duct with sperm to seminiferous duct without sperm were used. In present study for determine of these 3 index from every testis 200 cross sectional of seminiferous duct were studied and enumerated [37,49]. Values were represented as mean $\pm$ SEM. Data were analysed 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:

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 (figures 1&2). Histological studies of testicle 28 days after saffron prescription in 50ml/kg 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 (figure 3). Histological studies of testicle 28 days after saffron prescription in 100ml/kg dosage cleared that it results in considerable reduction in germinal cells number especially spermatide cells. There are sperms inside a few seminiferous ducts. Increasing the blood vessels crosses comparing control group is considerable (figure 4). Histological studies of testicle 28 days after saffron prescription in 200ml/kg 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



**Fig. 1:** Photomicrograph of seminiferous tubule in testis of rats after 28 day saffron administration with dosage of 100mg/kg (H&E×40).



**Fig. 2:** Photomicrograph of seminiferous tubule in testis of rats after 28 day saffron administration with dosage of 200mg/kg (H&E×400).

**Table 1:** Comparison the average of TDI, SPI and RI after orally administration of saffron with different dosages.

Parameter			
Groups	TDI (%)	SPI (%)	RI (%)
Control group	58/3±31/93	44/3±55/85	49/3±02/92
Saffron (50mg/kg)	65/4±5/94	46/4±62/89	33/5±90
Saffron (100mg/kg)	27/5±14/95	49/5±71/89	46/4±12/90
Saffron (200mg/kg)	79/2±83/61*	96/2±14/49*	23/1±85

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

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 (figure 5&6). 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 spermatozoid cells. 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 spermatozoid cells have been removed and there are just limited numbers of spermatogony. Testicle capsule doesn't show significant difference comparing previous groups (figures 6, 7& 8). From statistical analysis aspect for TDI in different groups

shows that; in dosages of 50 and 100 mg/kg after saffron administration TDI in comparison with control group a non significance increase shows but in group with dosage of 200mg/kg after saffron administration a significance reduce (p<0/05) in comparing with control group were observed. Also statistical analysis for RI and SI shows; in dosages of 50 and 100mg/kg after saffron administration RI in comparison with control group show non significance reduce but in dosage of 200mg/kg after administration this reduce is significance in comparison with control group (p<0/05), these results in table 1 has 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 spermicide 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 [38,39,40,44]. Saffron is also applied as a traditional anticancer drug on curing advanced prostate cancer due to its prevention effect on testosterone [38,44]. On a survey performed on mice, saffron consumption with 100mg/kg 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 [38]. In present study from SI, TDI and RI as index for determine spermatogenesis was used. Results of this study indicated that changes of these indexes in sub treatment rats group testicular tissue was with saffron administration with dosage of 200mg/kg and also the main changes in these indexes specially reduce of spermeiogenesis index in group 3 was for intensive reduce of spermatide and spermatozoa cells. Regarding the results of 50 and 100mg/kg 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 [38,40] 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 [33]. Based on Premkumar *et al* report, saffron due to its carotenoid compounds results in regulating the peroxidase activity of lipids, anti-oxidants and detoxification [47]. Takashi *et al* have reported that saffron's crocines through indirect increasing of m-R N A e n z y m e , g a m m a g l o t a m i l s y t e i n i l s y n t e t h a s e i n c r e a s e t h e r e s t o r e d g l u t a t h i o n e a m o u n t i n s i d e t h e c e l l a n d h a v e a c o n s i d e r a b l e i m p o r t a n c e i n r e g u l a t i n g t h e p e r o x i d a s e a c t i v i t y o f l i p i d s a n d a n t i - o x i d a n t s . S a f f r o n t h r o u g h e n h a n c i n g t h e d e f e n s i v e a n t i - o x i d a n t s y s t e m n o t o n l y r e d u c e s t h e o x i d a t i v e s t r e s s e s b u t a l s o i n c r e a s e s t h e l i f e t i m e a n d n u m b e r s o f s p e r m a t o s o i d s [12,39,51]. The conclusions of this research are also confirming saffron's popularity in traditional medicine as an enhancing the sexual power drug [3]. According to these results, saffron's extract can modify reproductive activities in male laboratory mice. It seems that the 100mg/kg/48h concentration of saffron has the most impact in this context [38,39]. 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 by Kuram Ali Ahmad 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 [31,38]. 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 [22]. 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 [3,38]. On the other hand, this study's results revealed that 200mg/kg 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 [38,39,44]. Previous studies supported the poisonous impact of saffron too. Researches have shown that appetite reduction is considered as a side effect following the treatment by saffron [43,45]. Mohajeri *et al* 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 [29,42]. Mohajeri *et al* 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 [42]. 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 [42]. A survey by Mohajeri *et al* displayed that high amounts of saffron (400mg) increases serum levels BUN and creatinine in humans [41]. Histopathological studies indicated that saffron causes considerable damages in liver and kidneys tissues among mice treated by saffron stigma total extract [42]. 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 [42]. 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 spermatogenesis and spermatogenesis indexes in rats. These results in amounts of TDI, SI and RI possibility causes by changes on seral concentration of testosterone. Of course the effect of saffron on spermatogenesis process and human infertility are needed to be more investigated.

### References

1. Abe, K., H. Saito, 2000. Effects of saffron extract and its constituent crocin on learning behavior and long-term potentiation. *Phytotherapy Res.*, 14: 149-52.
2. Abdullaev, F.I., 1993. Biological effects of saffron. *Bio Factors*, 4(2): 83-86.
3. Abdullaev, F.I., H. Caballero Ortega, L. Riveron Negrette, R. Pereda Miranda, J.M. Hernandez and J. Perez Lopez, 2003. use of in vitro assays to assess the potential genotoxic and cytotoxic effects of saffron (*Crocus sativus* L.). *Toxicology in Vitro*, 17: 731-736.
4. Abdullaev Jafariva, J., H. Caballero-Ortega, L. Riveron-Nigrete, R. Pereda-miranda, R. Rivera-Luna, J. Manuel Hernandez, I. Perez-Lopez, J.J. Espinosa-Aguirre, 2002. In vitro evaluation of chemopreventive potential of saffron.. *Rev. Inves. Clin.*, 54(5): 430-436.
5. Abdullaev, F.I., J.J. Espinosa-Aguirre, 2004. Biomedical properties of saffron and its potential use in Cancer therapy and chemoprevention trials. *Review, Cancer Detection and Prevention*, 28: 426-432.
6. Abdullaev, F.I., G.D. Ferenkel, 1992. Effects of saffron on cell colony formation and cellular nucleic acid and protein synthesis. *Biofactors*, 3: 201-204.
7. Abdullaev, F.I., L. RiveronNegrette, Rotenburd V. Belacortu, P.J. Kasurnov, I. Perez Lopez, J.M. Hernandez, J.J. Espinosa Aguirre, 2000. Saffron as chemopreventive agent. In: Wenyi T, Ed. *Food of 21<sup>st</sup> Century: Food and Resource Technology Environment*. China: Ligth Industry Press, 185-195.
8. Abolhasani, A., S.Z. Bathaie, I. Yavari, A.A. Moosavi-movahedi and M. Ghaffari, 2005. Separation and Purification of Some Components of Iranian Saffron. *Asian Journal of Chemistry*, 17(2): 727-729.
9. Akhondzadeh, S., H. Fallah-pour, K. Afkham, F. Khalighi, 2004. Comparison of crocus .lativlts L. and imipramine mild to moderate depression: A pilot double-blind [ISRCTN45683816] *Journal List BMC Complement Altern. Med.*, V.4.
10. Alonso, G.L., M.R. Salinas, J. Garijo, 1998. Method to determine the authenticity of aroma of saffron (*Crocus sativus* L.). *J. Food Prot.*, 61(11): 1525-1528.
11. Bisset, N.G., M. Wichtl, 2001. *Herbal Drugs and phytopharmaceuticals. A Handbook for Practice on a scientific Basis*, (2 ed.), Med. Pharm. Scientific publisher, CRC Press, London, 167-169.
12. Cao, L.C., S. Leers-Sucheta and S. Azhar, 2004. Aging alters the functional expression of enzymatic and non-enzymatic anti-oxidant defense systems in testicular rat Leydig cells. *The Journal of Steroid Biochemistry and Molecular Biology*, 88: 61-67.
13. Dellman, H.D. and J. Eurell, 1998. *Textbook of veterinary histology*, Fifth Edition, Williams and Wilkins, pp: 228-233.
14. De Smet, P.A.G.M., R. Hansel, R.F. Chandler, 1997. *Adverse Effect of Herbal Drugs*, Berlin, Springer-Verlag, 82.
15. Ebrahimzade, H., T. Rajabian, P. Abrishamchi, R. Karamian, A. Saboura, 2007. *Iranian Saffron from the Research View Point*, 1st ed. F.ttelat Publication, 587-591.
16. El-Daly, E.S., 1998. Protective effect of cysteine and vitamin E, *Crocus sativus* and *Nigella sativa* Extracts on cisplatin-induced toxicity in rats. *J. Pharm. Beig.*, 53(2): 87-95.
17. Escribano, J., *et al.*, 1996. Crocin, safranal and picrocrocin from saffron (*Crocus sativus*) inhibit the growth of human cancer cells in vitro. *Cancer Letters*, 100(1-2): 23-30.
18. Fatehi, M., T. Rashidabady and Z. Fatehi-Hassanabad, 2003. Effects of crocus sativus petals' extract on rat blood pressure and on responses induced by electrical field stimulation in the rat isolated vas deferens and guinea-pig ileum. *Journal of Ethnopharmacology*, 84: 199-203.
19. Fernandez, J.A., 2004. *Biology, Biotechnology and biomedicine of saffron: Recent Res Devel Plant Pci.*, 2: 127-59.
20. Gainer, J.I., J.R. Jones, 1975. The use of crocetin in experimental atherosclerosis. *Experientia*, 31: 548-549.
21. Garcia-Olmo, D.C., 1999. Effects of long-term treatment of colon adenocarcinoma with crocin, a carotenoid from saffron (*Crocus sativus*): an experimental study in the rat, *Nutrition and Cancer*, 35(2): 120-126.

22. Gopumadhavan, S., Mohamed Ra Fiq, M.V. Venkataranganna, Kala K. Suhas, S.K. Mitra, Assessment of 'Tentex royal' for sexual activity in an experimental model. *Indian J. ClinPrac.fiom*, 10(13): 23-6.
23. Hafez, B. and E.S.E. Hafez, 2000. Reproduction in farm animals, 7<sup>th</sup> Edition, Lippincott Williams and Wilkins, pp: 12-20.
24. Hosseinzadeh, H., V. Khesravan, 2002. Anticonvulsant effects of aqueous and ethanolic extracts of *Crocus sativus* L. stigmas in mice. *Arch Im. Med.*, 5: 44-7.
25. Hosseinzadeh, H., M.H. Modaghegh, Z. Saffari, 2007. *Crocus Sativus* L. (Saffron) Extract and its Active Constituents (Crocic and Safranolic) on Ischemia-Reperfusion in Rat Skeletal Muscle. Evidence-based Complementary and Alternative Medicine, 2007, Available at: <http://creativecommons.org/licenses/by-nc/2.0/uk/>.
26. Hosseinzadeh, H., H.R. Sadeghnia, T. Ziaee, A. Danaee, 2005. Protective effect of aqueous saffron extract (*Crocus sativus* L.) and crocin, its active constituent, on renal ischemia-reperfusion-induced oxidative damage in rats. *J. Pharm. Pharm. Sci.*, 8(3): 387-93.
27. Hosseinzadeh, H., F. Talebzadeh, 2005. Anticonvulsant evaluation of safranolic and crocin from *Crocus sativus* in mice. *Fitoterapia*, 76(7-8): 722-4.
28. Hosseinzadeh, H., H.M. Younesi, 2002. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. *BMC Pharmacol.*, 2: 7.
29. Karimi Gh., N. Taiebi, H. Hosseinzadeh, F. Shirzad, 2004. Evaluation of subacute toxicity of aqueous extract of *Crocus sativus* L. stigma and petal in rats. *Journal of Medical Plants*, Institute of Medical Plants, 12: 29-35.
30. Khorram, V., M. Naieb Pour, A. Mir Abbasi, E. Rakhshan, 2007. The Effect of aqueous extract of *Crocus sativus* on the basic and functional electrophysiological properties of isolated perfused rabbit AV-Nodal preparation. *Journal of Gorgan University of Medical Sciences*, 8(3): 1-7.
31. Kuram, A.H., B.V. Venkataraman, 1999. Assessment of a polyherbalayurvedic medicine for sexual activity in rats. *Indian Drugs*, 9(36): 576-82.
32. Li, N., G. Lin, Y.W. Kwan, D. Min, 1999. Simultaneous quantification of five major biologically active ingredients of saffron by high-performance liquid chromatography. *J Chromatogr*, A849(2): 349-355.
33. Lindi, L., C. Haolin, A. Michael, M.D. Trush, M. Show, D. Anway and R. Barry Zirkin, 2005. Aging and the Brown Norway Rat Leydig Cell Antioxidant Defense System. *Journal of Andrology*, 22: 32-37.
34. Loomis, T.A., 1968. *Essentials of Toxicology*. Philadelphia: Lea and Febiger, 67-78.
35. Lozano, P., D. Delgado, D. Gomez, M. Rubio, J.L. Iborra, 2000. A non-destructive method to determine the safranolic content of saffron (*Crocus sativus* L.) by supercritical carbon dioxide extraction combined with high-performance liquid chromatography and gas chromatography. *J Biochem Biophys Methods* 4328513-N (1-3): 367-378.
36. Martin, G., E. Goh, A.W. Neff, 2002. Evaluation of the developmental toxicity of crocetin on *Xenopus*. *Food and Chemical Toxicology*, 40(7): 959-964.
37. Meistrich, M., G. Wilson and K. Porter, 2003. Restoration of spermatogenesis in DBCP-treated rats by hormone suppression. *ToxicolSci*, 76(2): 418-426.
38. Modaresi, M., M. Mesripour, M. Asadi Margh Maleki, M.K. Hamedanian, 2008. Effect of Saffron (*Crocus Sativus*) extract on level of FSH, LH and testosterone in mice. *Journal of Zanjan University of Medical Sciences and Health Services*, 16(63): 11-17.
39. Modaresi, M., M. Mesripour, M. Asadi Margh Maleki, M.K. Hamedanian, 2008. The effect of Saffron extract on testis tissue. *Iranian Journal of Medicinal and Aromatic Plants*, 24(2): 237-243.
40. Modaresi, M., M. Mesripour, H. Nazern and M. Asadi, 2008. Effect of Saffron Extract on Pituitary- testis Axis in Mice. *International Journal of Health Science*, 1(1): 7-8.
41. Mohajeri, D., B. Amouoghli Tabrizi, Gh. Mousavi, M. Mesgari, 2008. Anti-diabetic activity of *Crocus sativus* L. (Saffron) stigma ethanolic extract in alloxan-induced diabetic rats. *Research Journal of Biological Science*, 3(9): 1102-1108.
42. Mohajeri, D., M. Mesgari Abbasi, A. Delazar, Y. Doustar, Gh. Mousavi, B. Amouoghli Tabrizi, 2009. Histopathological study of subacute toxicity of *Crocus sativus* L. (Saffron) stigma total extract on liver and kidney tissues in the rat. *Pharmaceutical Sciences*, 15(2): 115-124.
43. Moshiri, E., A. Akhondzadeh Basti, A.A. Noorbala, *et al.*, 2006. *Crocus sativus* L. (petal) in the treatment of mild-to-moderate depression: A double-blind, randomized and placebo-controlled trial. *Phytomedicine*, 13: 607-11.
44. Nair, S.C., S.K. Kurumboor, J.H. Hasegawa Saffron chemoprevention in biology and medicine: A review. *Cancer Biother.*, 10(4): 257-264.

45. Noorbala, A., S. Akhondzadeh, N. Tahmacebi-Pour, A.H. Jamshidi, 2005. Hydro-alcoholic extract of *Crocus sativus* L. versus fluoxetine in the treatment of mild to moderate depression: a double-blind, randomized pilot trial. *J. Ethnopharm.*, 97(2): 281-284.
46. Ochiai, T., H. Shimeno, K. Mishirna, *et al.*, 2007. Protective effects of carotenoids from saffron on neuronal injury invitro and invivo. *BiochimBiophysActa.*, 28: 1770(4): 578-84.
47. Prernkumar, K., S.K. Abraham, S.T. Santhiya and A. Ramesh, 2003. Protective effects of saffron (*Croccl.i. lativLLi* L.) on genotoxin-induced oxidative stress in Swiss albini mice. *Phytotherapy Research*, 17(6): 614-617.
48. Salomi, M.J., S.C. Nair, P.R. Panikkar, 1991. Cytotoxicity and non-mutagenicity of *Nigela saliva* and saffron (*Crocus .lativus*) in vitro. *Proc. Ker. Sci. Congr.*, 5: 244.
49. Shetty, G., G. Wilson and I. Huhtanieme, 2000. Gonadotropin-Releasing hormone analogs stimulated and testosterone ingibits the recovery of spermatogenesis in irradiated rats. *Endocrinology*, 141: 1735-1745.
50. Sunjay Kumar, G., K. Kala Suhas, 2002. Clinical evaluation oftentex royal in erectile dysfunction. *The Antiseptic*, 99(5): 161-2.
51. Takashi, O., S. Shinji, O. Shigekazu, T. Hiroyuki, Sh. Yukihiro and Sh. Hiroshi, 2004. Crocin prevent the death of PC-12 cells through sphingomyelinase- ceramide signaling by increasing glutathione synthesis. *Neurochemistry International*, 44: 321-330.
52. Tarantilis, P.A., M. Polissiou, M. Manfait, 1994. Separation of picrocrocin, cis-trans-crocins and safranal of saffron using high-performance liquid chromatography with photodiode-array detection. *J Chromatogr*, A664: 55-61.
53. Tarantilis, P.A., G. Tsoupras, M. Polissiou, 1995. Determination of saffron (*Crocus sativus* L.) components in crude plant extract using high-performance liquid chromatography-UV-visible photodiode-array detection-mass spectrometry. *J Chromatogr*, A699(1-2): 107-118.
54. Wuthrich, B., P. Schmid-Grendelmeyer, M. Lunberg, 1997. Anaphylaxis to saffron. *Allergy* 52(4): 476-477.