

Histological Effects of Aqueous Extract of Saffron Flowers *Crocus sativus* on Ovaries and Oviduct of Females White Mice *Mus musculus*

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Abstract

The present study was conducted to identify the histological effects of the aqueous extract of saffron flower on ovaries and oviducts of the adult female mouse at doses of 15, 30 and 60 mg/kg of body weight (b.w.) for the periods 7, 15 and 30 days, respectively and once a day. Behavioral changes were include the abnormal hyperactivity, irritability followed by introversion and reduction of motor activity, as well as loss of appetite for food and drinking water for several hours. The results showed histopathological changes of mice ovaries at a dose of 15 mg/kg of B.W. for the above periods a slight increase in primordial follicles, congestion of blood vessels, necrosis and degeneration. At a dose of 30 mg/kg. there was slight deposition of hyaline material, deposition of hemosiderin dye, congestion and increase of primordial follicles numbers, while at a dose of 60 mg/kg lymphatic cells infiltration were observed, with some ovarian stromal cells decaying, while the oviduct, and for the above periods changes included thickening of canal wall, endothelial cells degeneration and blood vessel congestion in the muscular area at a dose of 15 mg/kg, increased flattening of the mucosal folds also was observed. At a dose of 30mg/kg. an increased damage with increased blood vessels congestion, fibrosis of oviduct wall and degeneration of the external muscle layer of the oviduct at a dose of 60 mg/kg was obvious.

Introduction

The saffron plant is known as the scientific name *Crocus sativus* L. (Saffron). And that which belongs to the family of Iris. It's a widely used household spice because of its taste, color, and aromatic aroma, as well as its medical benefits. This plant contains various chemical compounds[1]. The plant is perennial plan, 10-30 cm long and the flower is the active part of the plant which used as seasoning after drying [2]. Saffron plants has medical benefits in the treatment of depression, blood pressure and inflammation, but it causes damage in different organs of the body if consumed irregularly, randomly and in large quantities because it contains chemical compounds such as carotenoids which are deformed factor (teratogen) [3]. The plant alcoholic extract also cause changes in weight and tissue at high doses, with various effects when injected in to the peritoneum of rats with doses of 0.70, 1.05mg/kg b.w. for 2-4 weeks [4]. LD50 of saffron is 200 mg/kg of b.w. in mice [1]. Several studies have been conducted to identify the effect of the saffron extract

on some vital organs in the body, Saffron extract was injected into the female rats with doses (2, 4) mg/kg for 10 days, the rats was to stimulated the formation of ovarian follicles, and increased number of secondary follicles[5]. Aromatic compound of saffron increase the secretion of estrogen in the mice within 20 minutes of eating the plant [6]. Saffron causes lack of movement and weakness [7]. Saffranal compound had a distinctly toxic effect when it was given to the mice orally with doses of 0.25, 0.1mg/kg for 21 days; caused irritation, inactivity of animals and changes in chemistry, such as low cholesterol and triglycerides levels in the bodies vital organs [8].

The present study was designed to investigate the histopathological changes in the ovaries and oviduct of adult females mice and to record the behavioral changes.

Materials and Methods

The present study was conducted on female adult mice aged 5-6 weeks with a mean weight of (28±2) gm. Mice were placed under appropriate laboratory

condition in term of ventilation, temperature of (26 ±2) C°, And photo cycle of 12 hours light: 12 darkness [9].

Saffron flowers purchased from local markets were used. The aqueous solution of the saffron was obtained by grinding dried flowers in the form of fine powder by an electrolytic mill. 60 mg of the powder was added with 500 ml of distilled water and left at room temperature for 24 hours and the solution was filtered using Buchner funnel and Wattman No.1 filter paper, The filtration solution dried using an oven at temperature of 45 C°. after drying distilled water was used to prepare the concentrations under study [10].

The experiments were designed using 60 female adult mice divided into four groups. The control group was administered orally with distilled water, while the other three experimental groups were administered orally with the aqueous solution of saffron flowers with doses of 15, 30, 60, mg/kg. for the periods 7,15,30 days and for one time per concentration used respectively. The females mice were dissected, and the ovaries and oviducts were fixed with formalin 10 % for 24 hours[11]. Histological sections were prepared depending on the method mentioned in the [11]. Samples were washed with distilled water and the dehydration process was carried out using progressive concentration of ethyl alcohol; The samples were also cleared and flooded with pure paraffin wax of melting point 56 – 57 C°. The molds were cut in thickness of 5 µm and were stained with Delafield's hematoxylin and eosin, sections were mounted with D.P.X., the sections were examined using optical microscope and for photographed using, a digital camera. The images were printed using a colored printer.

Results

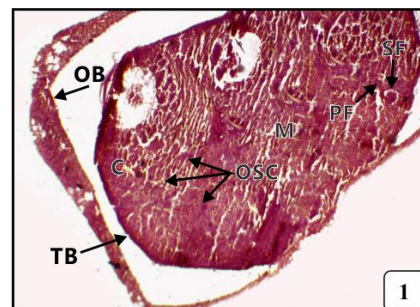
1. Behavioral changes:

The results of the study showed that , when the female mice were administered orally with aqueous extract of saffron, the symptoms of abnormal behavior at doses of 15, 30, 60 mg/ kg for 7 days except for slight excitation , either at the period of 15 days and the doses above were obvious. The of excitation period and excessive abnormal movement followed by calm and the convergence and reduction of motor events were noticed. The 30 days dose of the above concentrations showed loss of food and drinking water appetite for several hours and lower life activities than normal

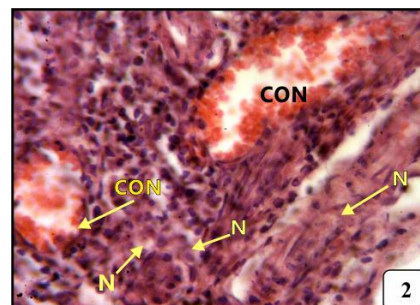
2. Histopathological changes in the ovaries

The microscopic examination of the adult female mouse ovaries showed, A group of control clarify the white tunica, the cortex, medulla, ovarian stromal cells, primary follicle, the bursa, they were all normal (P. 1). The results indicated that when the adult mouse was treated orally with aqueous extract of saffron plant with a dose of 15 mg/ kg for 7 days caused congestion of blood vessels, necrosis in the ovarian stromal tissue (P.2), while the

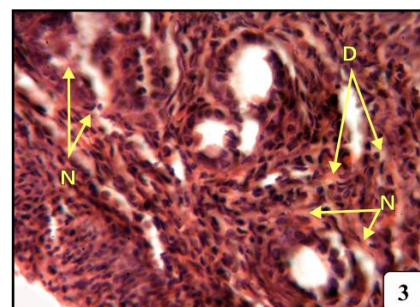
histopathological examination for 15 days showed cell degeneration and necrosis of some ovarian stromal cells (P.3). The 30 day dose increased the intensity of tissue lesions and was characterized by a slight increase in numbers of primary unilaminar follicles, degeneration and necrosis of the ovarian stromal cells (P.4). The histopathological changes of female mouse ovary at the dose of 30 mg / kg for 7 days indicated by the expansion and congestion of the blood vessel, degeneration of the fatty tissue surrounding the ovary and the deposition of hemosiderin, evidence of bleeding, and the appearance of macrophages (P.5). at 15 days period of treatment the number of primary follicles were increased and significant degeneration of ovarian stromal cells were observed (P.6).



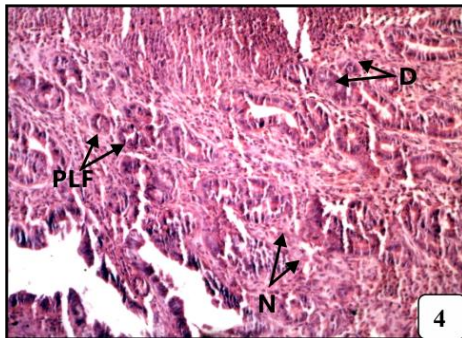
Picture (1): Histological section in a Female Ovary of adult mouse *Mus musculus* (control), showing tunica albuginea (TB), Cortex (C), medulla (M), ovarian stromal cells (OSC), Primary follicle (PF), secondary follicle (SF) & Ovarian bursa (OB). (H&E, 100X.)



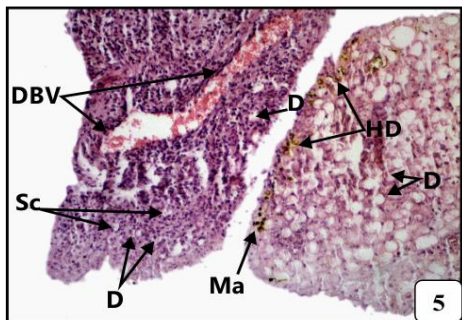
Picture (2): Histological section in a Female Ovary of adult mouse *Mus musculus* treated with the Aqueous Extract of Saffron at a dose of 15 mg /kg of b.w. for 7 days showing congestion (CON) of blood vessel (BV) & Necrosis (N) in the ovarian stromal cells (OSC). (H&E,400x)



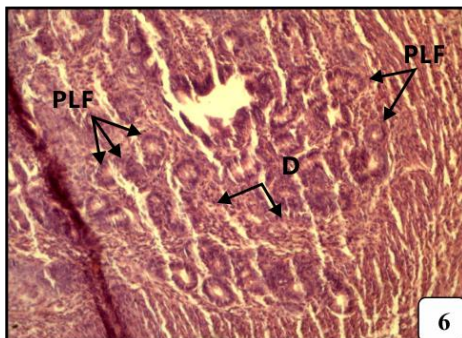
Picture (3): Histological section in a Female Ovary of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 15 mg /kg for 15 days shows Necrosis (N) & cellular degeneration (D). (H&E,400x)



Picture (4): Histological section in a Female Ovary of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 15 mg /kg for 30 days shows a slight increase of the primary follicles , Degeneration (D) & Necrosis (N) in some ovarian stromal cells (OSC). (H&E,100x)



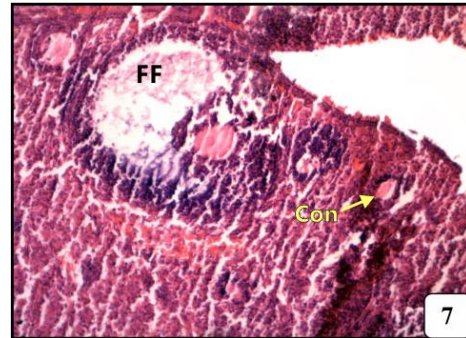
Picture (5): Histological section in a Female Ovary of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 30 mg /kg for 7 days shows Degeneration (D) in the fatty tissue surrounding the ovary & in some stromal cells (Sc), Dilatation of blood vessel (DBV), Hemosiderin deposition (HD), Macrophage (Ma). (100x, H&E).



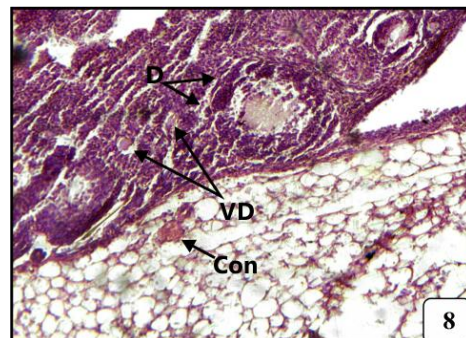
Picture (6): Histological section in a Female Ovary of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 30 mg /kg for 15 days shows the increasing in the primary laminar follicles (PLF) & Degeneration (D) in some stromal cells. (H&E,400X)

The results also showed an appearance of follicular fluid in the secondary multilayered follicle and congestion of blood vessels in the ovarian tissue (P. 7), While at the 30 day treatment, increased vitreous degeneration was observed in the secondary follicle and congestion of blood capillary (P.8). The histological examination of the female ovaries showed that the dose of 60 mg / kg for 7 days clarify the expansion of blood vessels, and degeneration of ovarian stromal cells which is took place around the

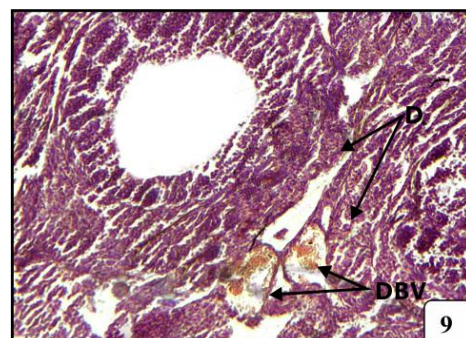
secondary follicle (P. 9). A significant infiltration of inflammatory lymphocytes also was observed (P.10). During the 15 days period, slight deposition of hyaline material was occurred in the primary and secondary ovarian follicles compared to previous concentration (P.11), also appearance of sever congestion of the blood vessel and acute infiltration of lymphocytic cells around the blood vessel (P. 12).



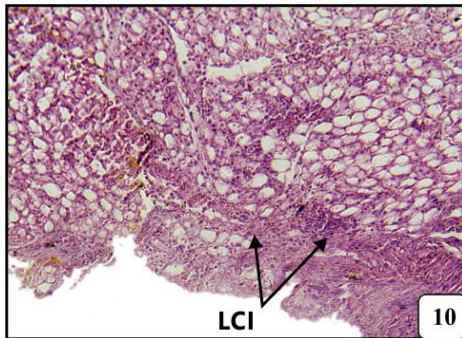
Picture (7): Histological section in a Female Ovary of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 30 mg /kg for 15 days shows Follicular Fluid (FF) in the Secodary follicule, Congestion (CON). (H&E,100X)



Picture (8): Histological section in a Female Ovary of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 30 mg /kg of b.w. for 30 days shows degeneration(D) vitreous degeneration (VD), Congestion (CON) of blood capillary. (H&E,100x).



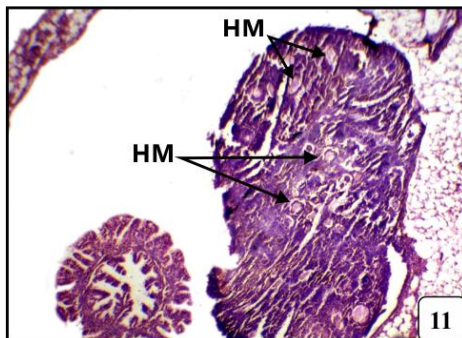
Picture (9): Histological section in a Female Ovary of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 60 mg /kg for 7 days shows Degeneration (D) of the stromal cells surrounding the secondary follicle & Dilatation of blood vessel (DBV). (H&E,100x)



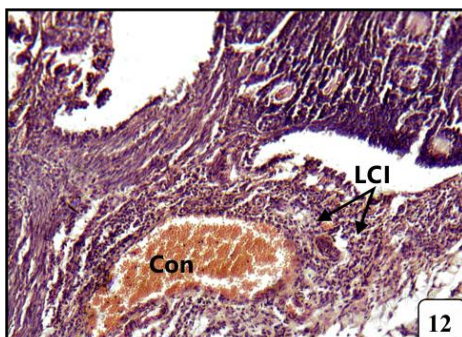
Picture (10): Histological section in a Female Ovary of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 60 mg /kg for 7 days shows Lymphatic cells infiltration (LCI) in the fatty tissue surrounding the ovary. (H&E,100x)



Picture (13): Histological section in a Female Ovary of adult mouse *M. musculus* treated with the the Aqueous Extract of Saffron at a dose of 60 mg /kg for 30 days shows loss of the cellular properties of the ovarian tissue (LCP) & decay of ovarian stromal cells (DC). (H&E, 100x)



Picture (11): Histological section in a Female Ovary of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 60 mg /kg for 15 days shows a slight Deposition of hyaline material (HM) in some secondary follicles (H&E,100x)



Picture (12): Histological section in a Female Ovary of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 60 mg /kg for 15 days shows congestion (CON) & lymphatic cells infiltration (LCI) around the blood vessel. (H&E, 100x)

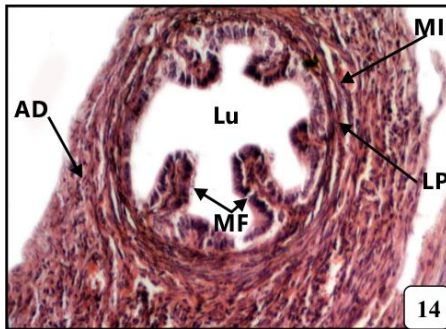
In the 30 day period, the loss of cellular properties of the ovary tissue and decay of the ovarian stromal cells. (P.13)

3. Histopathological changes in the oviduct

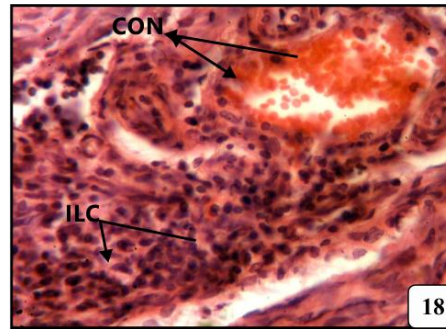
Microscopic examination of the adult mouse oviduct, control group showed the clarification of the adventitia, muscularies, lamina propria, lumen, mucosal folds& all were normal (P. 14).

The results with the adult female administered orally with aqueous extract of saffron at a dose of 15 mg / kg and for 7 days showed degeneration of the endothelial cells of the ovarian duct and congestion of the blood vessels (P. 15). The hyperplasia of the smooth muscle layer of the duct was observed at 15 days of treatment (P.16).

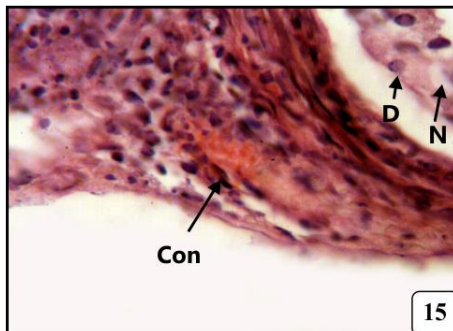
At the 30 days treatment the result showed ,the beginning of the flattening of the epithelial lining of the oviduct causes reduction of mucous folds (P, 17), while when treated with a dose of 30 mg /kg for 7 days the results showed, tissue lesion represented with increase of infiltration of inflammatory cells around the congested blood vessel (P.18). At 15 day the results showed, severe congestion of the blood vessel in the muscular layer of the oviduct (P, 19), while at 30 days of treatment the results showed the almost complete flatness of the epithelial folds (P. 20). At a dose of 60mg/kg for 7 days, the microscopic examination showed thickening of the oviduct wall, flattening of the endothelial lining of the duct and degeneration of cells compared with previous concentration with expansion of the blood vessel as well as gradual reduction of the lining of the duct (P.21). At day 15, sever thickening of muscle layer of the duct and sever congestion in the blood vessel were observed(P.22). At the 30 days period, increased severity of tissue lesion, the most important of which were fibrosis and totally loss of mucous folds of the duct (P.23).



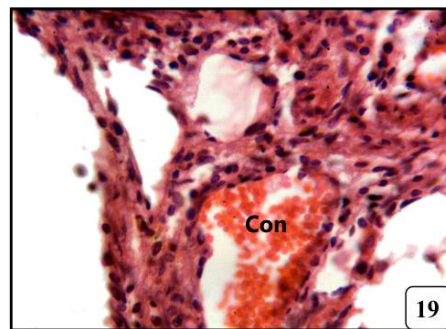
Picture (14): Histological section of Oviduct of adult mouse female *M. musculus* (control), showing adventitia (AD), Muscularis (MI), lamina propria (LP), lumen (LU), Mucosal folds (MF). (H&E, 100X)



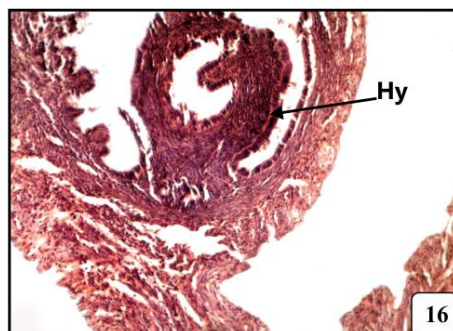
Picture (18): Histological section of Oviduct of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 30 mg /kg for 7 days shows increase of lymphatic cells infiltration (LCI) around the congested (CON) blood vessel. (H&E,400X).



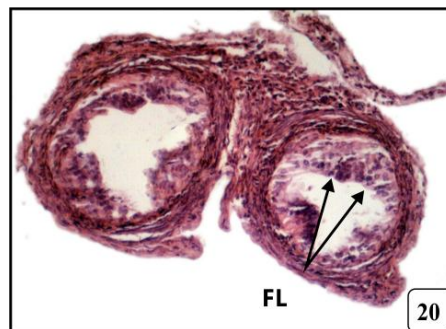
Picture (15): Histological section of Oviduct of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 15 mg /kg for 7 days shows Degeneration (D), Necrosis (N) in the epithelial lining & congestion (CON) of blood vessel (H&E, 400x).



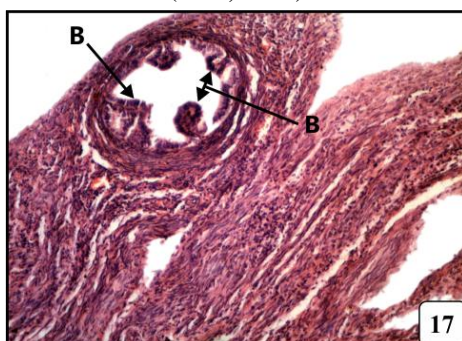
Picture (19): Histological section of Oviduct of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 30 mg /kg for 15 days shows severe congestion (CON) of blood vessel (BV) in the muscular layer of oviduct wall (H&E,400X)



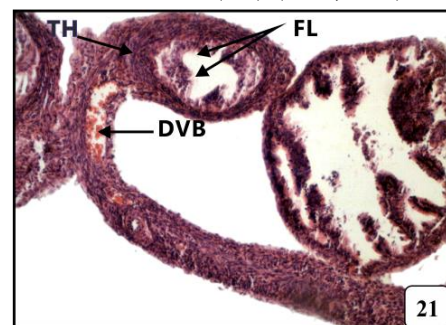
Picture (16): Histological section of Oviduct of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 15 mg /kg for 15 days shows Hyperplasia (HY) of the smooth muscle layer that forming the oviduct wall. (H&E, 100 X)



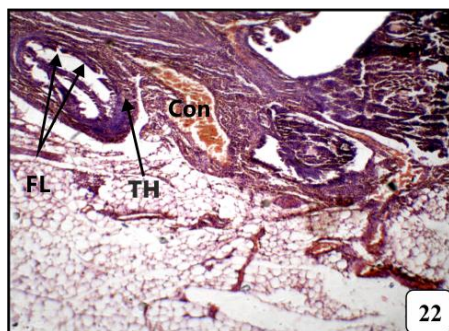
Picture (20): Histological section of Oviduct of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 30 mg /kg for 30 days shows a near-full flatness of the oviduct wall (FL). (H&E,100 X).



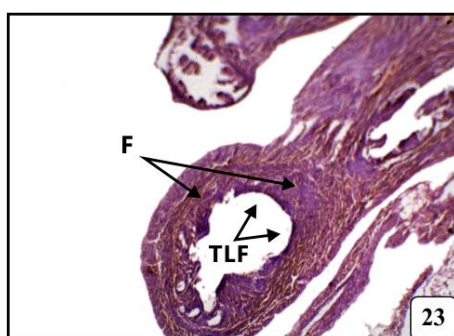
Picture (17): Histological section of Oviduct of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 15 mg /kg for 30 days shows the beginning of flattening of the internal epithelial layer (B) (H&E,100X)



Picture (21): Histological section of Oviduct of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 60 mg /kg for 7 days shows Thickening (TH) of the oviduct wall, dilation of the blood vessel (DBV) & Flattening (FL) of the mucous folds which lining the oviduct.. (H&E,100 X).



Picture (22): Histological section of Oviduct of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 60 mg /kg for 15 days shows severe thickening the oviduct wall (TH) , increase flattening (FL) of mucous folds , congestion (CON) of blood vessel in the muscular layer surrounding the oviduct (H&E, 100X).



Picture (23): Histological section of Oviduct of adult mouse *M. musculus* treated with the Aqueous Extract of Saffron at a dose of 60 mg /kg for 15 days shows Fibrosis (F) in the oviduct wall & Totally lost of folding (TLF). (H&E,100X)

Discussion

The present study showed abnormal behavioral changes which were represented with excitation followed by calm, contraction and reduction of motor activity of 30 days at dose of 60 mg/kg.

Results were consistent with what was indicated by the [8] when they treated the mice orally with saffranal at doses of 0.5, 0.1 ml/kg /d for 21 days causing irritation and inactivity of mice. While the results were not consistent with what [12] said the reason is that the aqueous extract of *Guiera senegalensis* plant reduces the spontaneous movement, reduce the period of sleep and activities of treated periods. The results also showed behavioral changes such as anorexia and low intake of food and water these changes are in line with those [13] have indicated by using intraperitoneal injection of aqueous extract of saffron in mice at doses of 10, 50, 100 mg/kg causing anorexia and low weight and the present results consistent with what [14] Through the intraperitoneal injection of saffron extract at doses of 40 -80 mg /kg , the lack of appetite in mice which treated with aqueous extract of the saffron plant in the present study may be due to given dose and the time of periods of the treatment. The reason for the lack of the kinetic activity for periods of treatment is due to the given dose as well as time of periods of the

treatment which caused the reduction of appetite and responsible for the lack of food consumption [15]. Overlap of the saffranal compound with GABA4 which is responsible for kinetic events [7]. while the results of the microscopic examination of the mice's ovaries showed satisfactory tissue changes when treated with doses of 15, 30, 60 mg /kg for 7,15,30 days was characterized by the congestion of blood vessel in the ovarian stromal tissue, necrosis, cellular degeneration, increase in number of ovarian follicles, degeneration of stromal cells, deposition of hemosiderin and the appearance of macrophages as well as the appearance of follicular fluid in the multilayered secondary follicle and increased the severity of tissue lesions by increasing degeneration in the tissue surrounding the ovary. The results of the present study agree with the increase in the numbers of germinal epithelial cells and primary follicles with what [5] pointed that, the alcoholic extract of saffron caused an increase in the number of primary and secondary follicles at peritoneal injection in female of rats at doses of 2, 4 mg/ kg for 10 days. The results of the present study are consistent with what the [9] recorded in the treatment of rats with alcohol extract of *Azadirachta excelsa* plant at a dose of 250 mg/kg of b.w. for 28 days in terms of congestion of the blood vessel in the ovaries and increase in the primary follicles numbers. while the present results do not agree with that [16] when said injecting the rats with oil extract of *Azadirachta indica* plant, will inhibit the formation of primary and secondary ovarian follicles, it may be attributed to the fact that the water extract of saffron contains a number of aromatic compounds that increase the secretion of estrogen and also matched by the discovery of some pathological tissue lesions such as hyalinization and necrosis as indicated by [17] when treating rats with different doses of the oil extract of *Artemisia annua* plant which resulted in satisfactory pathogens in the ovaries such as stromal cells necrosis and loose of connective tissue within the ovarian tissue, bleeding is also attributed to the deposition of hemosiderin and belevrine dye [18]. The results also showed the presence of inflammatory cells infiltration around the blood vessel in the ovarian white tunic, necrosis in the ovarian stromal cells and slight deposition of hyaline material in the primary and secondary follicles at a dose of 60 mg/ kg of the periods under study. This may be due to the fact that the aqueous extract of saffron stimulates the secretion of estrogen which in turn affects the efficiency of ovarian function as well as the occurrence of structural changes in the ovaries and ovarian follicles [9]. The results also indicated the presence of macrophages, fatty degenerate and a defect in a number of primary and secondary follicles, increasing at doses of 15 ,30 mg/ kg and their decay at other concentrations this may be due to the effect of aqueous extract of saffron on the amount of the estradiol excreted and the concentration of the follicular stimulating hormone

(FSH), and luteinizing hormone (LH) and which causes increase in numbers of primary and secondary follicles, especially saffron causes an increase in secretion of both LH and FSH [9].

The microscopic examination showed some histological changes in the oviduct of the mice females which treated with specific doses and periods under study and they were degeneration of the epithelial lining cells of the oviduct, decrease in the mucous folds of the inner lining of the oviduct, infiltration of inflammatory cells and flattening of the epithelial cells of the inner layer.

Histopathological lesions increased at a dose of 60 mg /kg with gradual reduction of epithelial folds, increased thickness of the oviduct wall, fibrosis and loss of mucous folds. The present results correspond to what was indicated by [9] in term of the appearance of thickening of the smooth muscles area of the myometrium near the oviduct and congestion of blood vessels in the treatment of mice with alcoholic extract of *Azadirachta excelsa* plant at a dose of 250 mg/kg. Results were consistent with [20] when he recorded the appearance of uterine hyperplasia when the rats were treated with the alcoholic extract of *Genetum africans* plant for three days and with doses

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of 100, 200, 700 mg/kg. The appearance of fibrosis in the present study is similar to what indicated by [9] when treating rats with alcohol extract of *Azadirachta excelsa* plant at a dose of 250 mg/kg for 28 days. present results result are also consistent with the occurrence of some tissue lesions, which were the reduction of the mucous folds lining the duct and hyperplasia of muscle layer with each of [21] when they treat mice with diethylstilbestrol with a dose of 10 microgram /dy for 5-7 months. flattening of epithelial layer cells, the progressive reduction of folds, their loss, and the severe congestion of the blood vessels in the oviduct tissue may be due to the fact that the extract under study contains aromatic and carotenoids compounds, which caused excessive excretion of estrogen which affects the levels of progesterone hormone, Thus affecting ovarian function and efficiency.

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التأثيرات النسجية للمستخلص المائي لأزهار نبات الزعفران *Crocus sativas* L. على مبايض وقناة

البيض لإناث الفئران البيض *Mus musculus*

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الملخص

اجريت الدراسة الحالية للتعرف الى التأثيرات النسجية للمستخلص المائي لأزهار نبات الزعفران *Crocus sativas* L. في مبيض وقناتي البيض في اناث الفأر البالغ عند التراكيز (15، 30، 60) ملغم/ كغم من وزن الجسم وللفترات الزمنية (7، 15، 30) يوماً لكل تركيز، على التوالي و مرة واحدة يومياً. تضمنت التغييرات السلوكية حركة مفرطة غير طبيعية والتهييج، اعقبها انزواء واختزال للفعاليات الحركية، فضلاً عن فقدان الشهية للطعام وشرب الماء لعدة ساعات. اظهرت النتائج تغييرات نسجية مرضية لمبايض الفئران وتمثلت عند التركيز 15 ملغم/ كغم من وزن الجسم وللفترات الزمنية اعلاه بازياد الخلايا الطلائية الجرثومية، واحتقان الاوعية الدموية، والنخر والتكس اما عند التركيز 30 ملغم/ كغم تمثلت بالتكس الزجاجي وترسب صبغة الهيموسيدرين، وتوسع الوعاء الدموي واحتقانه وازدياد اعداد الحويصلات الاولى، وعند التركيز 60 ملغم / كغم لوحظ ارتشاح الخلايا الالتهابية للمفاوية، اضمحلال بعض الخلايا الطلائية الجرثومية، اما قناة البيض فقد تمثلت التغييرات النسجية وللفترات الزمنية اعلاه ببتنخ جدار القناة، وتكس الخلايا البطانة واحتقان الاوعية الدموية في المنطقة العضلية عند التركيز 15 ملغم/ كغم، كما لوحظ ازدياد تسطح الطيات المخاطية. عند التركيز 30 ملغم /كغم، وازدادت حدة هذه الاضرار النسجية بازياد الاحتقان الدموي وتليف جدار القناة مع تكس لخلايا الطبقة العضلية الخارجية للقناة عند التركيز 60 ملغم / كغم من وزن الجسم.

الكلمات المفتاحية: الزعفران، مستخلص مائي، اضرار نسجية، مبيض، قناة بيض، فئران