Histopathological Effects Of Ethanolic Extract of Saffron Flowers

*Crocus sativus* L. On Stomach And Small Intestine of White Mice Females

*Mus musculus*

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**Abstract**

The current study was designed to identify the histopathological effects of the ethanolic extract of saffron flowers on the stomach and small intestine of female adult mouse at the doses of 25, 50 mg / kg of body weight (b.w.) for the periods 15, 30 days and once a day. Behavioral changes were included rapid movement of the animals, a simple lack of appetite for water and food, excitation, hyperactivity, hostility, weakness and convulsions in the movement of mice. The results showed histopathological changes of the mice stomach and that was at the dose of 25 mg / kg of b.w. for the above periods, lymphatic cells infiltration between gastric glands cells in the mucosa and submucosa, as well as degeneration and necrosis of the cells of the gastric glands and edema. While at the dose of 50 mg /kg of b.w. and for the same above periods, an increased lymphocytic infiltration with blood vessels congestion, degeneration and necrosis of a large number of cells in the muscularis mucosa were observed. Tissue lesions were increased as the period of treating increased, while the small intestine and for the above periods histological changes at the dose of 25 mg /kg b.w. characterized by congestion of blood vessels lining the small intestine, lymphocyte infiltration and its extension to the muscularis, degeneration of the villi epithelial cells and hemorrhage between muscularis cells. At the dose of 50 mg/kg of b.w. for the above periods, acute lymphocyte and neutrophils infiltration in mucosa and sub mucosa were observed, as well as sever degeneration in their cells, coagulative necrosis was also observed in the entire cells of the lining of the small intestine.

**Introduction**

Saffron is a spice derived from the flower of *Crocus sativus* L., family of Iridaceae, the plant is an autumn flowering perennial. Saffron has a long history of use in traditional medicine, Saffron has also been used as a fabric dye practically in china [1]. And the plant has benefits in the treatment of many diseases, including asthma, cough and many other diseases. Saffron is toxic when used at a dose higher than 2 grams daily and for a period of two weeks [2]. The toxic effects of saffron are likely attributable to the specific component of the essential oils, such as saffranal & protocrocin [3]. In mammals including humans and rats, the digestive tract is composed of tubular organs. It is known that digestion of a certain foods such as vegetables is often incomplete. It also known that while food is emptied from the mouth and esophagus as quickly as it is swallowed, emptying the stomach and small intestine in digestive process is relatively slow and not consciously controlled, for instance proteins are emptied slower than carbohydrate. The implication is that any toxic content of food may have very limited time to affect the mouth and esophagus, the potentials risk on the stomach or small intestine is impacted by the relative longer time stays in these two regions, therefore the stomach and small intestine are logically the regions of the digestive tract that may be most affected by any toxic component of foods [4].
The consumption of spices including saffron in high quantities cause damage to the tissues in the mucosa of gastrointestinal tract, because most of the spices contain oxidizing compounds such as phenylnproces, safroles, carotinoids which is also a teratogen [5,6]. Several studies have been conducted to investigate the toxic effect of the extract of saffron on vital organs in the body, including ovaries, liver, kidney in rats and mice, causing extensive tissue damage in the vital organs [7,8]. Due to the lack of studies the effect of the ethanolic extract of saffron on the histology of stomach and small intestine, therefore; the present study was undertaken to investigate the histopathological changes in the stomach and small intestine of the adult mice females and to record the behavioral changes induced by doses and time periods that have been used with ethanolic extract of saffron.

Methods and Materials

1. Extract Preparation:
Saffron flowers purchased from local markets were used. 100 gm. was grinded and converted to fine powder. The powder was mixed with 500 ml of ethanol 70 % and left to stand at room temperature for 48 hours. The plants active components these were filtered with cheese cloth and later with Wattman No.1 filter paper by suction and the filtrate was evaporated under vacuum at 40 C° until completely dried and the weight was recorded [9].

2. Experimental Animals and Treatment:
Fifty females Swiss albino mice weighing (23 ±2) gm were used. The females were divided in to 5 groups of 5 females each of them were isolated in plastic cages. All groups were exposed to a constant lab. Condition, temperature was about (25±2 C°), light /dark cycle of 12:12, fed with standard commercial diet and tap water. The first group of mice were orally administered with distilled water (control group).While the other four groups were orally administered with the ethanolic extract of saffron flowers at the doses of 25,50 mg /kg of B.W. for 15,30 days.

3. Histological Sections Preparation:
Adult mice were sacrificed, the stomach and small intestine were fixed in 10 % formalin, dehydrated in ascending series of ethanol alcohol, cleared in xylene and embedded in paraffin Sections and were cut at 5 μ thickness and stained with a double stain of hematoxylin–eosin [10], Sections were then mounted using D.P.X. and examined with optical microscope. For the photography a digital camera (Sony) was used. The magnification force was calculated by multiplying the objective lens factor. The images were printed using a color printer (Brother printer, LCBK / YC/M, Japan).

Results

1. Behavioral Changes:
The results showed that when the female of rats were treated with the ethanolic extract of saffron flowers, abnormal behavioral symptoms were observed at the dose of 25 mg / kg of B.W. for the period of 15 days, there was a rapid an abnormal movement of animals and, either in the period of 30 days the previous symptoms were increased and slightly with the observation of loss of appetite for the water drinking and the food, while the treatment of mice at the dose of 50 mg /kg of b.w. for 15 days increased excitation and hyperactivity after 15-20 minutes of administration and the symptoms were increased sharply during the period of 30 days represented by hostility and excessive movement followed by the aloneness in the corner of cage with increased weakness and convulsions in the movement of mice.

2. Histopathological changes in the stomach:
Microscopic examination of the adult mouse stomach of control group which administrated distilled water indicated, lamina properia (LP), gastric gland (GG), submucosa (SM), muscular layer (ML), serosa (Se), all were normal (Fig. 1).

Fig. (1): Histological section in a female stomach of adult mouse Mus musculus (control group), Shows lamina properia (LP), Gastric glands (GG), Submucosa (SM), Muscular layer (ML), Serosa (SE). (H & E. 100X)

The results indicated that when the adult females of mice were treated with ethanolic extract of saffron flowers at the dose of 25 mg /kg of b.w. for 15 days to tissue damage in the gastric lining represented with lymphatic cells infiltration around gastric glands in the mucosa and submucosa of the stomach wall. as well as a slight degeneration and necrosis of the gastric glands cells (Fig.2). While the tissue examination at the treatment for 30 days with the same dose above showed increased infiltration of lymphocytes between the cells of the gastric glands of the stomach mucosa ,also increased degeneration and necrosis of the cells compared to the previous period with the clarification of edema (Fig. 3).
The tissue lesions were increased at the dose of 50 mg/kg of b.w. for 15 days and were characterized by an increase in the lymphocytic infiltration between gastric glands, as well as degeneration and necrosis of a large number of mucosal cells observation (Fig. 4).

In the treatment for 30 days the infiltration of the lymphatic cells in the mucosa of the stomach were observed with spread of degeneration in the mucosa and sub mucosa with its extension to the muscularis layer (Fig. 5).

3. Histopathological Changes In The Small Intestine:
Microscopic examination of the adult female white mouse small intestine of the control group which treated with distilled water indicated: the serosa (Se), muscularia mucosa (MM), lamina properia (LP), intestinal villi (Vi), all were normal (Fig. 6).

The present results showed at the dose of 25 mg/kg of b.w. for the period of 15 days to a slight infiltration of lymphocytes and degeneration of some epithelial cells of gastric villi (Fig. 7). At the treatment for 30 days of the same dose above there was a marked increase in the infiltration of the lymphocyte in the mucosa of the small intestine extending into the muscularis, as well as the degeneration of many of its cells and congestion of its blood capillaries too, with a slight bloody hemorrhage in the mucosa layer were observed. (Fig. 8).
Doses above 50 mg/kg of b.w. for 15 days, Showed increases in lymphatic cells infiltration (LCI), degeneration (D) & necrosis (N) in the mucosa cells, (H&amp;E. 100X).

At the dose of 50 mg/kg of b.w. for 15 days, increased congestion in the blood vessels and increase in the lymphocyte infiltration were observed in the mucosa (Fig. 9). While at the period of 30 days for the same dose above, The previous lesions were exacerbated and characterized by sever infiltration of lymphocyte and neutrophils in the mucosa of the small intestine (Fig. 10), as well as sever degeneration and necrosis of mucosa cells and epithelial cells of the villi also was observed, compared to the previous doses and periods of treatment (Fig. 11).

Discussion
The present study recorded abnormal behavioral changes in females mice treated with the dose of 25 mg/kg of B.W. for 15 days which were a rapid movement and abnormally, as well the loss of appetite for water and food at the period of 30 days. These results do not agree with [11], while the results agreed with what [12] when they treated the mice with saffranal at the doses of 0.1, 0.5 ml/kg/day for 21 days causing irritation and inactivity. The results also showed that at the dose of 50 mg/kg of B.W. and for the periods of 15, 30 days, the above symptoms increased, including hyperactivity was noted, general weakness and after awhile there were convulsions in the movement of the animals. These results are consistent with what was indicated by [13] when they injected the mice intraperitoneally with saffron extract and at doses of 40-80 mg/kg of b.w. which cause general weakness & other symptoms, and also agree with what [14] when they injecting the mice intraperitoneally with aqueous extract of saffron at the doses of 10, 50, 100 mg/kg of B.W. and cause anorexia. The reason of the loss of appetite in the present study may be due to the fact that the ethanolic extract of saffron causes the reduction of the appetite which is responsible for lack food consumption [15], or may be due to the dose given and the time of periods, while the lack of the kinetic activity, general weakness and idle of the doses and the periods of the
saffron may be due to the essential oils, which they may have caused tissue damage in the stomach and its components [3]. The histological examination also revealed several tissue lesions in the small intestine of mice which treated with the ethanolic extract of saffron at the doses and time periods in the present study these lesions were represented with lymphatic cells infiltration, blood capillaries congestion in the mucosa and degeneration of the epithelial cells villi. The lesions were exacerbated by increased dose, duration of treatment and culminated at 50 mg/kg of b.w. for 30 days, sever degeneration and necrosis compared to previous doses and periods. These results were consistent with what [21] has pointed out when the guinea pigs was treated with the alcoholic extract of *Pittosporum ochrosaefolium* Bojer at dose of 46.69 mg/kg of b.w. this led to lymphatic cells infiltration, congestion of blood vessels in the mucosa and lamina properia of the small intestine. The results also agree with what [23] pointed out when the rabbits were treated with the leaf extract of *Calotropsis precrea* at the dose of 80 mg/kg of b.w. for 14 days, necrosis of mucosa and submucosa cells, degeneration of epithelial cells villi was observed. The results were similar to these which was indicated by [24] when the mice were treated with aqueous extract of *Phyllanthus amarus* at the doses of 400 - 800 mg/kg of b.w. for 30 days, it caused lymphatic cells infiltration in the duodenum and distortion of small intestine cells were observed . In the present study, the reason of the lesions may be due to the consumption of saffron in a high quantities which causes tissue damage, especially in the gastrointestinal tract because that most spices including saffron contain oxidizing substances such as safrols, carotinoids, phenylpropanes, which cause extensive tissue damage when it was used for long term use . In the present study the effects of saffron ethanolic extract may be due to the given dose and time periods. Actually the reason for gastrointestinal damage is that the food stays in it longer than the rest of the digestive parts. There for, these two organs (stomach & small intestine) logically they seems to be the most affected by any toxic compound that many food contains and this includes all the foods which passes thought it [25].

References


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التأثيرات المرضية النسيجية للمستخلص الإيثانولي لازهار نبات الزعفران على Crocus sativus L. مع معدة الفئران البيض Mus musculus  

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المؤسسات:

صممت الدراسة الحالية لتشخيص التأثيرات المرضية الديفية للمستخلص الكحولي لأزهار نبات الزعفران على المعدة والإمعاء الدقيقة لإنسان الفأر البالغ بعد الجرعة 25، 50 ملغ / كغم من وزن الجسم والفترات 15، 30 يوما و لكل تركيز، شملت التغيرات السلوكية، الحركة السريعة للحيوانات، فقدان شعور للذوق للشريحة للماء والطعام، ابهيج، نقص حركة، عدائية، ضعف و تشنجات في حركة الفأر. أظهرت النتائج تغيرات مرضية لمعدة الفأر وتمثّلت عند الجرعة 25 ملغ / كغم من وزن الجسم والفترات أعلاه بارتياح الخلايا الالتهابية المفيدة بين الغدد المعدية في الطبقة المخاطية تحت المخاطية للمعدة، فضلا عن التوك و نخر الغدد المعدية والوذمة، أما عند التركيز 50 ملغ / كغم من وزن الجسم والفترات الزمنية ذاتها فلم يحدث ازدياد الارتشاح للخلايا الالتهابية مع احتقان الأوعية الدموية في المنطقة العضلية المخاطية و نخر و نخر عدد كبير من الخلايا في الطبقة المخاطية و تحت المخاطية للمعدة. وازدادت الاضرار شدة بزيادة الفترة الزمنية للمعالجة. أما الإعاء الدقيق فقد تمثلت التغيرات السريرية عند التركيز 25 ملغ / كغم من وزن الجسم والفترات أعلاه بارتياح الخلايا الالتهابية و احتقان الأوعية الدموية و انتقاد الوذمة في المنطقة العضلية و ضعف الخلايا الطلائية لزغات و الانتفاخ في الطبقة العضلية، أما عند الجرعة 50 ملغ / كغم من وزن الجسم والفترات أعلاه فلم يحدث الارتشاح الحاد للخلايا الالتهابية و انتقاد الوذمة في المنطقة المخاطية. فضلا عن تكو ان و خدر حنيد لخلايا م عوم خلايا بطاقة الامعاء.

الكلمات المفتاحية: زعفران، مستخلص إيثانولي، أمراض نسجية، معدة، إمعاء دقيقة، فئران