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Nephrotoxicity and hepatotoxicity evaluation of *Crocus sativus* stigmas in neonates of nursing mice

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ABSTRACT

Background: *Crocus sativus*, known as saffron crocus, is best known for the spice saffron. Saffron use spans more than 3500 years, however, its toxicity on neonates during lactation has not yet evaluated.

Objectives: This study was aimed to examine the acute toxicity of saffron on adult mice and its nephrotoxicity and hepatotoxicity on neonates of lactating mothers that used saffron during lactation.

Materials and Methods: In this experimental study, following acute toxicity evaluation, 32 pregnant mice were randomly designated into four equal groups. Following delivery, the mothers of groups 1 to 4 were administered orally (by gavage) normal saline (control group), 500, 1000 or 2000 mg/kg/day of saffron for three weeks, respectively. The newborns' kidney and liver parameters were assessed at the end of the study for possible nephrotoxicity and hepatotoxicity evaluation. The kidney and liver tissue samples of newborns were histopathologically studied after staining with Hematoxylin & Eosin. Data were analyzed using ANOVA and Scheffe's tests

Results: The LD_{50} value of saffron was calculated to be 4120±556 mg/kg in mice. To evaluate lactating toxicity, saffron was administered orally to the mothers once daily for 21 days, after delivery, during lactating period. Saffron increased serum urea nitrogen ($p< 0.05$). Histological studies indicated that saffron did not have any toxic effect on liver, however, histopathology changes were seen in the kidney of neonates.

Conclusions: From the results of present study, it might be concluded that saffron is a nearly safe spice, however, nursing mothers should avoid high doses of this spice.

Original Article

Implication for health policy/practice/research/medical education:
Saffron prepared from Crocus sativus is a nearly safe spice, however, nursing mothers should avoid high doses of this spice. The data suggest that administration of high doses of saffron to mothers, in sub-acute evaluation might damage the infants' kidneys.

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1. Introduction

Medicinal plants have long been used or recently evaluated for different diseases, especially for hard curable conditions such as Alzheimer (1,2), atherosclerosis (3,4), diabetes mellitus (5,6), cancer (7,8) and gastrointestinal diseases (9,10). Although these plants are considered to be safe, however, they may cause damage due to their unwanted side effects (11,12). Therefore, studying the side effects of medicinal plants would have

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effective role in identifying and diagnosis of safety profile if these drugs in human.

Crocus sativus, known as saffron crocus, is a flowering plant from Crocus genus (Iridaceae family) which is best known for the spice saffron, produced from its flowers (Figure 1). Saffron is one of the world's most costly spices (13). The cultivation of *Crocus sativus* and saffron use spans more than 3,500 years. *Crocus sativus* grows up to about 12 inches and bears three to four flowers, each with three vivid crimson stigmas. The dried stigmas are used mainly as coloring agent. *Crocus sativus* was originated in Greece and is native to Southwest Asia especially Iran (13).

The saffron taste results from the chemicals safranal and picrocrocin. It also contains crocin which imparts a rich golden-yellow color to dishes (14). Nowadays, Iran accounts for about 90 % of the world production and trade of saffron. Saffron is widely used in Iranian, Indian, European, Turkish and Arab cuisines and confectioneries. Saffron substitutes include safflower *Carthamus tinctorius* (safflower) and *Curcuma longa* (turmeric) (13).

Saffron has many potential medicinal properties and a long history of medicinal use as part of traditional healing. Recent research studies have suggested that saffron has preventive effects on cancer and cancer mutation. It has also immunomodulating, antioxidant and antidepressant activities. Saffron also protects the eyes from the direct effects of bright light and retinal stress apart from slowing retinitis pigmentosa and macular degeneration. Male subjects use saffron for prevention of premature ejaculation or infertility and women use it for premenstrual syndrome or menstrual cramps. Saffron is also used for cough, pertussis, asthma, heartburn, insomnia, hardening of arteries, flatulence, Alzheimer's disease, shock, hemoptysis, pain, alopecia, dry skin, as an aphrodisiac and expectorant (15).

Figure 1. Saffron, its flower and the Saffron crocus field

The physiological action of saffron is not well-understood yet. Although saffron has been used for a long time in different countries with no reported clinical toxicity and seems to be safe, however, few investigations have been conducted on its toxicological profiles. Recently, in a clinical trial 1.2 g saffron petal induced diarrhea, bleeding, nausea and vomiting (16) and at 200 mg/kg/day changed some biochemical parameters in normal ranges, in healthy volunteers (17).

2. Objectives

This study was aimed to evaluate the pathological effects of different doses of saffron on kidney and liver parameters in the newborns of mice which their mothers were exposed to saffron during the lactating period.

3. Materials and Methods

In this experimental study saffron, prepared from *Crocus sativus,* was purchased from a grocery in Mashhad city in Khorasan province, Iran. The dried saffron was pulverized using mechanical mill and dissolved in hot water. For evaluation of acute toxicity (LD_{50}), 32 Balb/C mice weighing 30±2 g and aging about 12 weeks, purchased from Pasteur Institute of Tehran were used. The animals were kept in laboratory animals in a normal condition with 21±2 °C temperature, and floor covering standards with unlimited water and food. To evaluate acute toxicity, animals were designated into 4 equal groups and were administrated by gavage 750, 1500, 3000 and 6000 mg/kg doses of saffron dissolved in distilled water. The animals were observed for 24 hours for death status. For the first 8 hours their neurological symptoms were observed in two-hour intervals. The mortality rate was measured and the LD_{50} was determined using Wilcoxon and Litchfield method (18).

To evaluate the nephrotoxicity and hepatotoxicity

of saffron in neonates of lactating mothers, 32 female Balb/C mice of about 12 weeks old and weight range of 30±2 grams were employed. Each two female mice were placed in a cage with a male mouse for two nights for mating. Pregnancy was diagnosis by observing the vaginal plug and positive vaginal smear. Then the pregnant mice were randomly divided into three experimental and a control groups.

Animal experiments were approved by the Ethical Committee of Shahrekord University of Medical Sciences, Iran. The animals were administered saline, 500, 1000 or 2000 mg/kg/day of saffron from the delivery day until the end of lactating period. A day after the last dose, cardiac blood sampling was collected, after making the animals anesthetized, in order to conduct biochemical studies.

After blood sampling, the neonate mice were killed by cervical dislocation and their livers and kidneys were completely removed and fixed in 10% buffered formalin to determine pathology changes.

Sections of 3µ diameter were prepared after obtaining paraffin blocks and then were transferred to slides and were stained by Hematoxylin & Eosin stain. After confirming the normal distribution of the data, they were analyzed by one-way ANOVA and in case of significant difference between the experimental and control groups, Scheffe's test was used. $P< 0.05$ was considered as significantly different.

4. Results

4. 1. LD_{50} value evaluation

Approximately 4 min following administration of saffron, the animals became excited and exhibited hyperactivity which continued for not more than 5 min. Then the animals became sedated and their locomotor activities, food and water consumption decreased which were more significant at higher doses. The LD_{50} value of saffron in mice was calculated to be 4120±556 mg/kg.

4.2. Effects of saffron on neonates of lactating mothers

Nephrotoxicity and hepatotoxicity effects of saffron in neonates of lactating mothers were evaluated following administration of saffron (by gavage) for 21 days with 500, 1000 and 2000 mg/kg/day. None of these doses showed any significant difference in aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, serum creatinine and albumin (Table 1). The level of serum urea nitrogen (BUN) in 1000 and 2000 mg/kg/day dose groups of animals decreased (Table 1).

4.3. Effect of saffron on histopathological parameters of newborn mice

The histopathology examination of the kidney and liver tissue sections of newborn mice in the control group did not show any structural and pathological changes. The histopathology of neonates' livers in the experimental group which their mothers received different doses of saffron and the neonates' kidneys which their mothers received lower dose of saffron also revealed no structural changes.

In newborns of the experimental group that received 1000 and 2000 mg/kg/day of saffron, the histopathology study of the kidney tissues showed interstitial nephritis accompanied by mononuclear inflammatory cell infiltration around the renal tubules, particularly around the renal arteries and the presence of eosinophilic protein-rich fluid within renal tubules. In experimental groups which received 2000 mg/kg/day dose, these observations were more pronounced and was along with presence of glomeruli disappearance in some areas of the cortical region.

5. Discussion

The LD_{50} of saffron in mice was calculated to be 4120±556 mg/kg. Based on the toxicity classifications, any substance with LD_{50} value within the range of 1 to 5 g/kg practically is usually considered as low-toxic and any substance with LD_{50} bellow 5 g/kg may be considered practically

Table 1. Effect of different doses (mg/kg/day) of saffron on serum biochemical parameters in mice treated for three weeks

Variable Parameter	Saline Control	5000	1000	2000
BUN (mg/dL)	37.43 ± 3.2	35.16 ± 2.51	58.5 ± 4.99*	70.31 ± 13.30**
Creatinine(mg/dL) (mg/dL)	0.41 ± 0.03	0.44 ± 0.04	0.37 ± 0.02	0.37 ± 0.1
ALT (IU/L)	67.36 ± 7.01	71.21 ± 6.41	58.16 ± 3.04	68.03 ± 8.06
AST (IU/L)	87.66 ± 9	99.33 ± 7.94	117.5 ± 14.24	123.4 ± 40.15
ALP (IU/L)	63.05 ± 8.23	62.61 ± 5.52	55.3 ± 4.94	58,65 ± 4.06
Total bilirubin(mg/dL) (mg/dL)	0.42 ± 0.01	0.39 ± 0.02	0.39 ± 0.03	0.38 ± 0.03

Data are shown as Mean± SEM (n =8). *p < 0.05, **p < 0.01, compared with control group. BUN: blood urea nitrogen, ALT: alanine aminotransferase, AST: aspartate aminotransferase, and ALP: alkaline phosphatase.

non-toxic (19,20). The calculated LD_{50} value of saffron in mice was in the range of 1-5 g/kg. Thus, saffron should practically be considered as low toxic in acute evaluation.

Medicinal plants usually have various components which may possess potential of causing useful and/or harmful effects. To evaluate beneficial effect of saffron, other than assessing the acute and sub-acute toxicities, we need to assess its toxic effects in pregnancy and lactation, too. Due to variations in systems of detoxification and differences in absorption, distribution, excretion mechanisms in different animal species, there are species differences in response to toxic substances (18). Therefore, the toxic effects in human should be considered with these differences.

In lactating toxicity study (three weeks evaluation), saffron at doses of 500, 1000 or 2000 mg/kg/day did not cause any death. However, animals which received saffron showed evidence of hyperactivity and excitation which might be related to the irritant nature of most essential oils. Following this excitation, saffron showed sedative effect and reduction of loco motor activity. Saffron has been shown to have an agonistic activity on $GABA_A$ receptors (21), hence, its hypnotic and anti-anxiety effects seem to be related to this interaction (22).

In this study, serum urea nitrogen was increased. Histopathology evaluations of the kidney showed some changes and abnormalities. However, there were no changes on the histopathology and common markers of liver toxicity such as ALT, AST and bilirubin. Thus, it might be presumed that saffron has no significant toxic effect to liver, but at high doses might cause kidney damage to the lactating neonates of mothers that use saffron during lactation.

The levels of BUN and creatinine are appropriate indicators of renal function. The animals treated with saffron showed an increase in blood urea nitrogen (BUN). The increased level of BUN in the treated groups might be attributed to renal damage and this is further confirmed by the kidneys' histopathology findings.

6. Conclusions

In conclusion, from the results of the present study on the acute and sub-acute toxicities of saffron, it might be concluded that saffron prepared from *Crocus sativus* is a nearly safe spice, however, nursing mothers should avoid high doses of this spice. The data suggest that administration of high doses of saffron to mothers, in sub-acute evaluation might damage the infants' kidneys. Further studies are necessary to evaluate more these results.

Author contributions

All authors wrote the manuscript equally.

Conflict of interests

The authors declared no competing interests.

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References

1. Rabiei Z, Rafieian-kopaei M, Heidarian E, Saghaei E, Mokhtari S. Effects of zizyphus jujube extract on memory and learning impairment induced by bilateral

electric lesions of the nucleus basalis of meynert in rat. Neurochem Res 2014;39(2):353-60.
2. Rabiei Z, Rafieian-Kopaei M, Mokhtari S, Alibabaei Z, Shahrani M. The effect of pretreatment with different doses of Lavandula officinalis ethanolic extract on memory, learning and nociception. Biomed Aging Pathol 2014; 4:71-76.
3. Mohammadifard N, Sarrafzadegan N, Sajjadi F, Maghroun M, Khosravi A, Alikhasi H, et al. Potato consumption and cardiovascular disease risk factors among Iranian population. Int J Food Sci Nutr 2012; 63(8):913-20.
4. Nasri H, Sahinfard N, Rafieian M, Rafieian S, Shirzad M, Rafieian-kopaei M. Effects of Allium sativum on liver enzymes and atherosclerotic risk factors. J HerbMed Pharmacol 2013; 2(2):23-28.
5. Kafash-Farkhad N, Asadi-Samani M, Rafieian-Kopaei M. A review on phytochemistry and pharmacological effects of Prangos ferulacea (L.) Lindl. Life Sci J 2013; 10(8s):360-367.
6. Mirhoseini M, Baradaran A, Rafieian-Kopaei M. Medicinal plants, diabetes mellitus and urgent needs. J HerbMed Pharmacol 2013; 2(2):53-54.
7. Shirzad H, Shahrani M, Rafieian-Kopaei M. Comparison of morphine and tramadol effects on phagocytic activity of mice peritoneal phagocytes in vivo. Int Immunopharmacol 2009; 9(7-8):968-70.
8. Shirzad H, Taji F, Rafieian-Kopaei M. Correlation between antioxidant activity of garlic extracts and WEHI-164 fibrosarcoma tumor growth in BALB/c mice. J Med Food 2011; 14(9):969-74.
9. Sedighi M, Rafieian-kopaei M, Noori-Ahmadabadi M. Kelussia odoratissima Mozaffarian inhibits ileum contractions through voltage dependent and beta adrenergic Receptors. Life Sci 2012; 9(4):1033-8.
10. Kiani MA, Khodadad A, Mohammadi S, Ghayour Mobarhan M, Saeidi M, Jafari SA, *et al.* Effect of peppermint on pediatrics' pain under endoscopic examination of the large bowel. J HerbMed Pharmacol 2013; 2(2):41-44.
11. Rafieian-Kopaei M. Medicinal plants and the human needs. J HerbMed Plarmacol 2012; 1(1):1-2.
12. Nasri H, Shirzad H. Toxicity and safety of medicinal plants. J HerbMed Plarmacol 2013; 2(2): 21-22.
13. Mazhari N. Iridaceae. In: Asadi M, editor. Flora of Iran. Vol. 31. Tehran: Research Institute of Forests and Rangelands; 2000. pp. 4–6.
14. Daniel M. Medicinal Plants: Chemistry and Properties. Enfield: Science Publishers; 2006. pp. 138.
15. Javadi B, Sahebkar A, Emami SA. A survey on saffron in major Islamic traditional medicine books. Iran J Basic Med Sci 2013; 16(1):1–11.
16. Schmidt M, Betti G, Hensel A. Saffron in phytotherapy: Pharmacology and clinical uses. Wien Med Wochenschr 2007; 157(33):315-9
17. Hosseinzadeh H, Shariaty MV, Khadem-Sameni A, Vahabzadeh M. Acute and sub-acute toxicity of crocin, a constituent of Crocus sativus L. (saffron), in mice and rats. Pharmacologyonline 2010; 2:943-51.
18. Piyachaturawat P, Tubtim C, Chuncharunee A, Komaratat P, Suksamrarn A. Evaluation of the acute and subacute toxicity of a choleretic phloracetophenone in experimental animals. Toxicol Let 2002; 129:123-32.
19. Loomis T. Essential of Toxicology. Philladelphia:Lea and Febiger;1968. 67-78.
20. Kennedy GL, Ferenz RL, Burgess BA. Estimation of acute oral toxicity in rats by determination of the approximate lethal dose rather than the LD. J Appl Toxicol 1986; 6:145-8.
21. Hosseinzadeh H, Sadeghnia HR. Protective effect of safranal on pentylenetetrazol-induced seizures in the rat: involvement of GABAergic and opioids systems. Phytomedicine 2007; 14(4):256-62.
22. Hosseinzadeh H, Noraei NB. Anxiolytic and hypnotic effect of Crocus sativus aqueous extract and its constituents, crocin and safranal, in mice. Phytother Res 2009; 23:768-74.