Evaluation of teratogenic effects of crocin and safranal, active ingredients of saffron, in mice

Seyed Adel Moallem, Mohammad Afshar, Leila Etemad, Bibi Marjan Razavi and Hossein Hosseinzadeh

Toxicol Ind Health published online 4 October 2013
DOI: 10.1177/0748233713500818

Toxicology and Industrial Health
1–7

DOI: 10.1177/0748233713500818
tih.sagepub.com
SAGE

Evaluation of teratogenic effects of crocin and safranal, active ingredients of saffron, in mice

Seyed Adel Moallem[1,2], Mohammad Afshar[3,2], Leila Etemad[4], Bibi Marjan Razavi[4,5] and Hossein Hosseinzadeh[1]

Abstract

Saffron (*Crocus sativus*) is a widely used food additive for its color and taste. Crocin and safranal are two main components of this plant. Numerous studies are underway to introduce saffron and its active ingredients as pharmacological agents. Safety assessments of these compounds are important parts of this endeavor. In this study, the effects of crocin and safranal administrations during embryogenesis have been investigated in mice. A total of 75 BALB/c pregnant mice were divided into six experimental and control groups. Four experimental groups received intraperitoneal injection of crocin (200 mg/kg or 600 mg/kg) daily or safranal (0.075 ml/kg or 0.225 ml/kg) on gestational days (GDs) 6 to 15. Control groups received normal saline or paraffin as solvents of crocin and safranal. Dams were dissected on GD18 and embryos were collected. Routine maternal and fetal parameters were recorded. Macroscopic observation of external malformations was also performed. Fetuses were then selected for double skeletal staining with alizarin red and alcian blue. All experimental groups caused significant decrease in length and weight of fetuses when compared with the control groups and revealed malformations such as minor skeletal malformations, mandible and calvaria malformations, and growth retardation. Minor skeletal malformations were the most commonly observed abnormality, which were statistically significant when compared with the control groups ($p < 0.05$). The severities of malformations were comparable in the crocin- and safranal-treated groups. This study suggests that crocin or safranal can induce embryonic malformations when administered in pregnant mice. Due to the wide use of saffron, further elaborate studies to understand the malformation mechanisms of these ingredients are recommended.

Keywords

Saffron, *Crocus sativus*, crocin, safranal, teratogenicity, mice

Introduction

Crocus sativus L. (Iridaceae), commonly known as saffron, is a perennial stemless herb of the Iridaceae family, widely cultivated in Spain, Iran, and other countries (Soeda et al., 2007). It has been used as a flavoring and coloring agent for many thousands of years. In traditional medicine, it is believed that saffron has several properties such as antispasmodic, eupeptic, anticatarrhal, nerve sedative, carminative, diaphoretic, expectorant, stimulant, stomachic, aphrodisiac, and abortion(Dehghani and Dehghani, 2009; Moghaddasi, 2010).It has been used to treat snoring, toothache, otitis, anal pain, and gout (Hosseinzadeh and Nassiri-Asl, 2013).Also, modern pharmacological studies have demonstrated that saffron has antioxidant, anticancer, anticonvulsant, anti-inflammatory and antitumor

[1]Department of Pharmacodynamics and Toxicology, Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Department of Anatomy, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran
[4]Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[5]Faculty of Health, Sabzevar University of Medical Sciences, Sabzevar, Iran

Corresponding author:
Hossein Hosseinzadeh, Department of Pharmacodynamics and Toxicology, Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad 91775-1365, Iran.
Email: hosseinzadehh@mums.ac.ir

effects, radical-scavenging as well as learning- and memory-improving properties (Abdullaev and Espinosa-Aguirre, 2004; Hosseinzadeh and Talebzadeh, 2005; Hosseinzadeh et al., 2004).The pharmacological activities of saffron are attributed to its active constituents such as volatile agents (e.g., safranal), bitter principles (e.g., picrocrocin), and dye materials(e.g., crocetin and its glycoside, crocin) (Basheeruddin Asdaq and Inamdar, 2010).

Crocin is a carotenoid isolated from saffron and is responsible for the red color of saffron. It is a pharmacological active component of saffron. Modern pharmacological studies have demonstrated that crocin can be used as a new therapeutic agent. It has antitumor activity, antioxidant, radical scavenging properties, hypolipemic, and memory-improved effects (Soeda et al., 2007; Zheng et al., 2007).

Safranal (deglycosylated picrocrocin) is one of the three major constituents of *C. sativus* that is responsible for the characteristic aroma of saffron (Zougagh et al., 2006). It has been reported that safranal possesses anticonvulsant activity (Hosseinzadeh and Sadeghnia, 2007) and antidepressant (Hosseinzadeh et al., 2004) and antioxidant effects (Assimopoulou et al., 2005). Also, it can protect the brain against ischemia (Hosseinzadeh and Sadeghnia, 2005).

In spite of the wide use of saffron as a flavoring and coloring agent and for its medicinal value, little is known about its effect on embryonic development if used by pregnant mothers. Few animal studies reported that high concentrations of the aqueous extract of saffron can produce embryonic abnormalities (Tafazoli et al., 2004). Research on crocetin, another carotenoid isolated from saffron, has shown that this compound acts as a teratogen (Martin et al., 2002).

Due to the wide use of saffron and its active components in traditional medicine and modern pharmacology and the belief by many that saffron is a safe food and medicinal agent, it is estimated that many young women may utilize it during their pregnancy. Therefore, studying the teratogenicity of saffron and its components should be performed.

In this study, the embryotoxicity of crocin and safranal as a result of exposure during organogenesis has been examined in mice.

Material and methods

Material and animal treatment

Crocin and safranal were obtained from Fluka Chemical Company (St Gallen, Switzerland). Alizarin red and alcian blue were purchased from Merck (Darmstadt, Germany). BALB/c mice (aged 10–12 weeks and weighing 20–30 g weight) were obtained from the animal house of the Avicenna Research Institute, Mashhad University of Medical Sciences, Mashhad, Republic of Iran. They were kept under natural dark/light schedule (12-h light/dark cycles) and room temperature (18–22°C) at least for 2 weeks prior to mating. All animal experiments were approved by the Animal Care Committee of Mashhad University of Medical Sciences. They received standard laboratory diet and water *ad libitum*. Females were paired with a male of the same stock in a 2:1 ratio overnight and examined for a vaginal plug the following morning. The vaginal plug formation observed in the morning was considered as the 0th day of gestation (GD0) (Tian et al., 2005). Pregnant mice were divided into six groups. Each group consisted of 15 and 10 pregnant mice in crocin- and safranal-treated groups, respectively. The first and second groups were considered as control and received intraperitoneal (IP) injection of normal saline and paraffin as solvents of crocin and safranal, respectively. Groups 3 and 4 received 200 and 600 mg/kg/day of IP crocin, respectively. Groups 5 and 6 received IP injection of 0.075 and 0.225 ml/kg/day of safranal, respectively. All injections were administered daily during GDs 6 to15 (organogenesis period). For dose selection, median lethal dose and maximum tolerated doses (MTD) of crocin and safranal were determined and 10 and 30% of MTDs were chosen as the administered doses.

Maternal observation

Maternal body weights were recorded on GD0 through GD18. All pregnant females were observed daily throughout gestation for mortality, morbidity, general appearance, and behavior.

Fetus observation and staining

The pregnant mice were euthanized on day 18 of gestation. The uteri were removed and weighed. Macroscopic evaluations including appearance, number of fetuses, number of live and dead fetuses, weight and length of body of fetuses, and resorption sites were carried out. Microscopic observation of external malformations (exencephaly, cleft palate, abdominal hernia, polydactyl, open eyelid, etc.) under a dissecting microscope was performed. Malformed fetuses were then selected to detect skeletal anomalies by a specific double staining. Some control fetuses and the malformed

Table 1. Macroscopic fetal parameters in crocin and control groups.[a]

	Control	Crocin (200mg/kg)	Crocin (600mg/kg)
Number of pregnant mice	15	15	15
Number of total fetuses	182	167	165
Number of live fetuses	182	160	148
Mean pregnant mice weight ±SEM	34.6 ± 0.5	33.8 ± 1.2	35.7 ± 0.47
Number of resorption sites (%)	0	7 (4.2%)[b]	17 (10.3%)[b]
Mean fetal length ± SEM	23.6 ± 1.9	20.04 ± 2.57[b]	20.46 ± 2.68[b]
Mean fetal weight ± SEM	1.22 ± 0.8	1.094 ± 0. 26	1.026 ± 0.20[c]
Mean of fetal total number ± SEM	12.13 ± 1.8	11.13 ± 2.28	11 ± 4.12

IP: intraperitoneal; GD: gestational day.
[a]Pregnant mice received 200 and 600 mg/kg of IP administration of crocin during GD6–15. Control received saline. Data are expressed as mean ± SEM.
[b]$p < 0.001$ when compared with the control.
[c]$p < 0.05$ when compared with the control.

fetuses were fixed in 90% ethanol for 3 days and then were stained with alizarin red and alcian blue as reported with some modifications (Inouye, 1976; Kimmel and Trammell, 1981). Images of skeletal anomalies were recorded using a stereomicroscope (model MZ 12, Leica, Solms, Germany).

Statistical analysis

The data on weight and crown–rump of fetuses were expressed as mean ± SEM and tested with analysis of variance followed by Tukey–Kramer's multiple comparison test. Differences in frequencies of fetal malformations and resorptions were assessed either using χ^2 or Fisher's exact tests.

Results

Effect of crocin and safranal on maternal and fetal parameters in mice

All fetuses were removed by cesarean section on GD18. During the pregnancy period, food and water consumption of pregnant mice did not differ significantly among the different groups. Behavior signs in dams of either doses of crocin or safranal did not change, and there were no notable changes in the number of fetuses in both treated groups when compared with their respective control groups.

The number of live fetuses, weight and length of body of embryos, and resorption sites are shown in Tables 1 and 2. In crocin-treated mice, there was a significant increase in resorption sites in both groups as compared tothe control group ($p < 0.001$). Also, the resorption rate was significantly increased at the dose level of 600 mg/kg when compared with 200 mg/kg group ($p < 0.05$). The mean weight of fetuses was significantly decreased in the 600 mg/kg group when compared with the control ($p < 0.05$). Moreover, there was a significant reduction in the mean body length of fetuses in both treated groups when compared with the control group ($p < 0.001$; Table 1).

Safranal at both doses caused significant decrease in mean fetal bodyweight compared to their respected control groups ($p < 0.05$). Reduction in mean fetal body length was significant in both treated groups when compared with the control ($p < 0.001$). However, there was no significant difference in mean fetal body weight and length between the two treated groups. The number of the resorption sites increased significantly in both groups ($p < 0.001$; Table 2).

Comparison of gross fetal abnormalities following exposure to crocin and safranal

Incidence rates and types of malformations detected in fetuses treated with crocin and safranal are shown in Tables 3 and 4, respectively. The percentages of malformed fetuses were 9.4 and 6.8% in mice at doses of 600 and 200 mg/kg of crocin, respectively, which was significantly higher when compared with the control group ($p < 0.001$; Table 3).

The incidence of minor skeletal malformations such as wavy ribs and sternal anomalies was more than the other observed malformations. The percentage of these skeletal malformations was significantly increased at doses 200 and 600 mg/kg of crocin when compared with the control (5 vs. 5.4%, respectively; $p < 0.05$). As shown in Table 3, mandible and calvaria

Table 2. Macroscopic fetal parameters in safranal and control groups.[a]

	Control	Safranal (0.075ml/kg)	Safranal (0.225ml/kg)
Number of pregnant mice	10	10	10
Number of total fetuses	130	132	128
Number of live fetuses	130	129	120
Mean pregnant mice weight ± SEM	33.6 ± 0.9	34.1 ± 0.4	35.23 ± 0.5
Number of resorption sites (%)	0	3 (2.27%)[b]	8(6.25%)[b]
Mean fetal length ± SEM	22.07 ± 1.9	20.14 ± 2.45[b]	20.07 ± 2.63[b]
Mean fetal weight ± SEM	1.22 ± 0.8	1.06 0. 30[c]	1.02 ± 0.32[c]
Mean of fetal total number ± SEM	13 ± 1.8	13.2 ± 2.28	12.8 ± 1.12

IP: intraperitoneal; GD: gestational day.
[a]Pregnant mice received 0.075 and 0.225 ml/kg of IP administration of safranal during GD6–15. Control received paraffin. Data are expressed as mean ± SEM.
[b]$p < 0.001$ when compared with the control.
[c]$p < 0.05$ when compared with the control.

malformations and growth retardation were observed in the treated groups. However, the rate of these malformations was not significantly elevated when compared with the control (Table 3).

In safranal-treated mice, the results show that the occurrence of anomalies in the treated groups was significantly higher when compared with the normal fetuses (10.8 and 15.8% in the 0.075 and 0.225 ml/kg, respectively) (Table 4). Some gross malformations of fetus in mice treated with safranal and crocin were presented in Figure 1. Similar to the crocin-treated mice, the most marked abnormality observed in different doses of safranal-treated groups was minor skeletal malformations. The differences in total minor skeletal malformations between control and safranal groups were significant. However, incidence rates of mandible and calvaria malformations, curly tail, and limb deformity were not significantly elevated. Two to three embryos in the treated litters appeared markedly growth retarded.

Discussion

The purpose of this study was to evaluate the potential effects of crocin and safranal acting as teratogens in pregnant mice. Results showed that administration of 200 and 600 mg/kg of crocin to pregnant mice on GD6–15 produced embryotoxicity as indicated by the decrease in weight and length of fetuses and increase in resorption sites in a dose-dependent manner. Moreover, safranal caused a reduction in body weight and length of fetuses and increased resorption sites. Martin et al. (2002) have evaluated the teratogenic potential of crocetin (another active component of saffron) on Xenopus. In agreement with our results, they found that crocetin is a teratogen and produces a reduction in length of fetuses when compared with the control (Martin et al., 2002). Moreover, the results of one placebo-controlled trial indicated that Satiereal consumption, a novel extract of saffron stigma, can lead to body weight loss by reducing appetite and snacking (Gout et al., 2010).

It was stated that saffron can induce abortion and increase the risk of maternal death at high doses (Dehghani and Dehghani, 2009). Previous studies have demonstrated that aqueous extract of saffron increases uterine contractions in rat and guinea pig (Abdullaev and Espinosa-Aguirre, 2004). In this study, two preterm deliveries were observed in the dose level of 600 mg/kg of crocin on GD16. This effect may be related to direct stimulation of saffron and its constituents on uterus smooth muscle due to increase in prostaglandins. Also, it was shown that the use of saffron in the first trimester of pregnancy in humans increases the level of prostaglandins in uterus and leads to abortion.

Our results showed that administration of crocin and safranal during organogenesis produced several malformations (Tables 3 and 4). The incidence of skeletal malformations was more than the other observed malformations. Other anomalies, which have been shown following safranal and crocin administrations, include mandible and calvarias malformation, curly tail, limb deformity, and growth retardation. It was reported that aqueous extract of saffron (100 mg/kg) produced osteogenesis retardation in mice. It is likely that saffron may inhibit the growth and proliferation of osteoblasts (Golalipour et al., 2008). The higher rate of skeletal malformations in this study may be due to the effect of saffron and its components on

Table 3. Incidence and types of fetal malformations following treatment with crocin.[a]

	Control	Crocin (200 mg/kg)	Crocin (600 mg/kg)
Number of pregnant mice	15	15	15
Number of live fetuses	182	160	148
Minor skeletal malformations	0	8 (5%)[b]	8 (5.4%)[b]
Mandible and calvariamalformation	0	2 (1.25%)	3 (2.03%)
Growth retardation	0	1 (0.62%)	3 (2.03%)
Number of total malformations	0	11 (6.88%)[c]	14 (9.46%)[c]

IP: intraperitoneal; GD: gestational day.
[a]Pregnant mice received 200 and 600 mg/kg of IP injection of crocin during GD6–15. Control received saline. Data are expressed as mean ± SEM.
[b]$p < 0.05$ when compared with the control.
[c]$p < 0.001$ when compared with the control.

Table 4. Incidence and types of fetal malformations following treatment with safranal.[a]

	Control	Safranal (0.075 ml/kg)	Safranal (0.225 ml/kg)
Number of pregnant mice	10	10	10
Number of live fetuses	130	129	120
Minor skeletal malformations	0	6 (4.65%)[b]	8 (6.67%)[b]
Mandible and calvaria	0	2 (1.55%)	3 (2.5%)
Limb deformity	0	3 (2.32%)	4 (3.33%)
Curly tail	0	1 (0.77%)	1 (0.83%)
Growth retardation	0	2 (1.55%)	3 (2.5%)
Number of total malformations	0	14 (10.85%)[c]	19 (15.83%)[c]

IP: intraperitoneal; GD: gestational day.
[a]Pregnant mice received 0.075 and 0.225 ml/kg of IP injection of safranal during GD6–15. Control received saline. Data are expressed as mean ± SEM.
[b]$p < 0.0500$ when compared with the control.
[c]$p < 0.001$ when compared with the control.

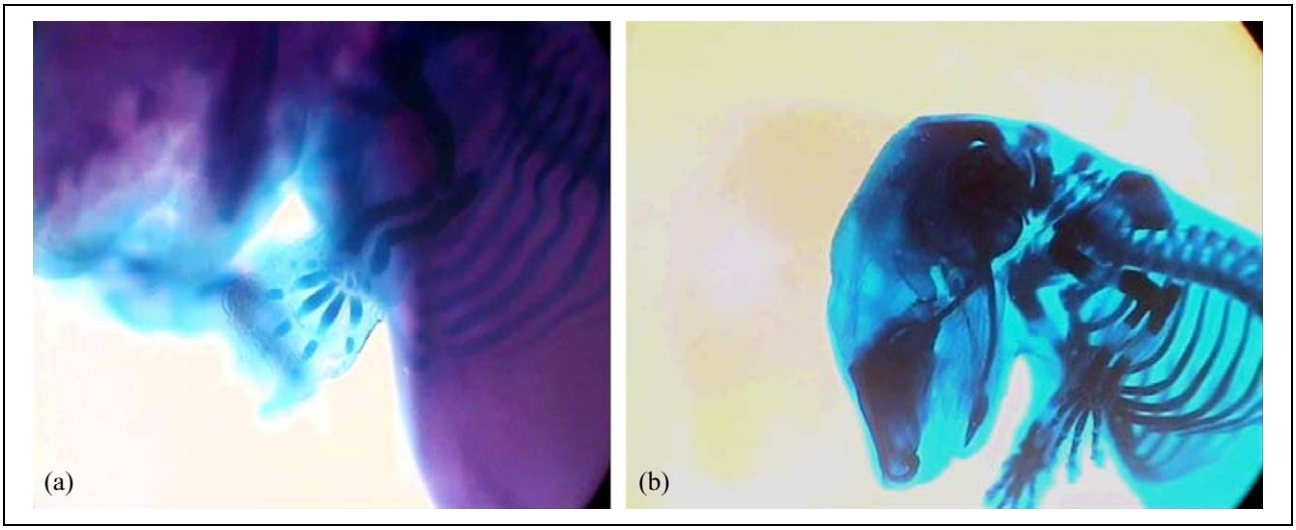

Figure 1. Fetuses with some malformations in the upper limb shown as deformities in radius, ulna, and phalanx in safranal-treated mice (GD6–15) at the 0.225 ml/kg dose (a) and hypoplasia of mandible in the crocin (600 mg/kg) group (b). GD: gestational day.

bone formation or skeletal growth during organogenesis. According to our findings, crocin and safranal, like crocetin, at two selected doses caused a significant increased risk of total and skeletal malformations. The mechanisms of teratogenicity of saffron and its components are not known. One of the mechanisms of saffron as an anticancer agent is attributed to the inhibition of synthesis of DNA and RNA, which in turn inhibit cell proliferation (Abdullaev and Espinosa-Aguirre, 2004). Since coordination of cell proliferation and cell death is essential to attain normal embryonic development, saffron may thus interfere with normal growth and produce malformations. It is well known that saffron is safe, and oral administration of saffron extract at concentrations of up to 5 g/kg has shown to be nontoxic in mice. However, the use of this plant in doses higher than 5 g/kg produced different adverse effects in human subjects such as heart rate reduction, nausea, and abortion (Abdullaev and Espinosa-Aguirre, 2004). Our study has presented the possibility of saffron teratogenicity. Therefore, great caution in saffron use during pregnancy is necessary.

Further studies are recommended to explore the teratogenicity mechanism of saffron and its main components.

Conclusion

The results showed that if the main components of saffron, crocin and safranal, are used during organogenesis, they can adversely affect the growth of fetuses and induce several fetal malformations, noticeably skeletal malformations. Great caution should be taken into consideration when saffron is used during pregnancy.

Acknowledgment

The authors thank Dr Fatemeh Shirzad for her technical assistance in carrying out this project.

Funding

The present work was financially supported by the Vice Chancellor of Research, Mashhad University of Medical Sciences, Mashhad, Republic of Iran.

References

Abdullaev FI, Espinosa-Aguirre JJ (2004) Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials. *Cancer Detection and Prevention* 28(6): 426–432.

Assimopoulou AN, Sinakos Z and Papageorgiou VP (2005) Radical scavenging activity of *Crocus sativus* L. extract and its bioactive constituents. *Phytotherapy Research* 19(11): 997–1000.

Basheeruddin Asdaq SM, Inamdar MN (2010) Potential of *Crocus sativus* (saffron) and itsconstituent, crocin, as hypolipidemic and antioxidant in rats. *Applied Biochemistry and Biotechnology* 162: 358–372.

Dehghani Z, Dehghani S (2009) Effect of saffron on the increasing of abortion. In: *International Symposium on Saffron: A Herbal Medicine of 3rd Millennium*, May 13–14, Mashhad, Iran.

Golalipour MJ, Gharravi AM, Ghafari S, Afshar M and Khori V (2008) Effects of *Crocus sativus* on the fetal development of NMRI mice. *Saudi Medical Journal* 29(2): 309–310.

Gout BC, Bourges C and Paineau-Dubreuil S (2010) Satiereal, a *Crocus sativus* L. extract, reduces snacking and increases satiety in a randomized placebo-controlled study of mildly overweight, healthy women. *Nutrition Research* 30(5): 305–313.

Hosseinzadeh H, Karimi G and Niapoor M (2004) Antidepressant effect of *Crocus sativus* L. Stigma extracts and their constituents, crocin and safranal, in mice. *Acta Horticulturae* 650: 435–445.

Hosseinzadeh H, Nassiri-Asl M (2013) Avicenna's (Ibn-Sina) the canon of medicine and saffron (*Crocus sativus*): a review. *Phytotherapy Research* 27: 475–483.

Hosseinzadeh H, Sadeghnia HR (2005). Safranal, a constituent of *Crocus sativus* (saffron), attenuated cerebral ischemia induced oxidative damage in rat hippocampus. *Journal of Pharmacy and Pharmaceutical Sciences* 8(3): 394–399.

Hosseinzadeh H, Sadeghnia HR (2007) Protective effect of safranal on pentylenetetrazol-induced seizures in the rat: involvement of GABAergic and opioids systems. *Phytomedicine* 14(4): 256–262.

Hosseinzadeh H, Talebzadeh F (2005) Anticonvulsant evaluation of safranal and crocin from *Crocus sativus* in mice. *Fitoterapia* 76(7–8): 722–724.

Inouye M (1976) Differential staining of cartilage and bone in fetal mouse skeletal by alcian blue and alizarin red s. *Congenital Anomalies* 16(3): 171–173.

Kimmel CA, Trammell C (1981) A rapid procedure for routine double staining of cartilage and bone in fetal and adult animals. *Stain Technology* 56(5): 271–273.

Martin G, Goh E and Neff AW (2002) Evaluation of the developmental toxicity of crocetin on Xenopus. *Food and Chemical Toxicology* 40(7): 959–964.

Moghaddasi MS (2010) Saffron chemicals and medicine usage. *Journal of Medicinal Plants Research* 4(6): 427–430.

Soeda S, Ochiai T, Shimeno H, Saito H, Abe K, Tanaka H, et al. (2007) Pharmacological activities of crocin in saffron. *Journal of Natural Medicines* 61(2): 102–111.

Tafazoli M, Kermani T and Saadatjoo A (2004) Effects of saffron on abortion and its side effects on mice Balb/c. *Ofogh-e-Danesh* 10(3): 53–56.

Tian Y, Ishikawa H, Yamaguchi T, Yamauchi T and Yokoyama K (2005) Teratogenicity and developmental toxicity of chlorpyrifos. Maternal exposure during organogenesis in mice. *Reproductive Toxicology* 20(2): 267–270.

Zheng YQ, Liu JX, Wang JN and Xu L (2007) Effects of crocin on reperfusion-induced oxidative/nitrative injury to cerebral microvessels after global cerebral ischemia. *Brain Research* 1138: 86–94.

Zougagh MA, Rois A and Valcarcel M (2006) Determination of total safranal by in situ acid hydrolysis in supercritical fluid media: application to the quality control of commercial saffron. *Analytica Chimica Acta* 578(2): 117–121.