Chapter 34

Safety and toxicity of saffron

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Chapter Outline

34.1 Introduction

Saffron is the most expensive dietary spice and is derived from the stigmas of *Crocus sativus* L. Most of this herbal medicine is produced in Iran, mainly in South Khorasan province (Alavizadeh and Hosseinzadeh, 2014; Shahi et al., 2016). The taste and odor of this spice is due to picrocrocin and safranal, while the red color is due to the crocin (Alavizadeh and Hosseinzadeh, 2014; Shahi et al., 2016). The carotenoids of saffron are sensitive to light, heat, oxygen, and enzymatic oxidization (Hosseini et al., 2018a).

Saffron has a long history of use in traditional medicine (Hosseinzadeh and Nassiri-Asl, 2013; Mollazadeh et al., 2015). In various studies, saffron and its main constituents have exhibited different valuable properties (Alavizadeh and Hosseinzadeh, 2014; Hosseini et al., 2018b; Hosseinzadeh and Jahanian, 2010; Hosseinzadeh et al., 2008; Khazdair et al., 2015; Shafiee et al., 2017).

Experimental studies have confirmed the positive effects of saffron and its active ingredients in cardiovascular disorders (Broadhead et al., 2016; Imenshahidi et al., 2010), convulsion (Hosseinzadeh and Talebzadeh, 2005; Khazdair et al., 2015), depression (Broadhead et al., 2016; Khazdair et al., 2015; Vahdati-Hassani et al., 2014), Alzheimer's (Alavizadeh and Hosseinzadeh, 2014; Broadhead et al., 2016; Khalili and Hamzeh, 2010), cancer (Aung et al., 2007; Abdullaev and Espinosa-Aguirre, 2004), metabolic syndrome (Doumouchtsis et al., 2018), renal failure (Boozari and Hosseinzadeh, 2017), and digestive disorders (Khorasany and Hosseinzadeh, 2016).

Additionally, the therapeutic effects of saffron in depression (Shafiee et al., 2017), Alzheimer's (Akhondzadeh et al., 2010a), and diabetic maculopathy (Sepahi et al., 2018) have been reported in clinical studies. Interestingly, saffron and its bioactive ingredients have been mentioned as protective or antidote components against natural or chemical toxins (Razavi and Hosseinzadeh, 2015). The protective effects of these agents against potent toxins including bisphenol A (Vahdati-Hassani et al., 2017), diazinon (Lari et al., 2013; Razavi et al., 2013a,b), malathion (Mohammadzadeh et al., 2018), acrylamide (Mehri et al., 2015), cumene hydroperoxide (Yousefsani et al., 2018), and doxorubicin (Chahine et al., 2016; Elsherbiny et al., 2016) have been confirmed.

Although saffron-based compounds have been extensively investigated in pharmacological, clinical, and toxicological aspects, little is known about the safety of these agents in vivo (Badie-Bostan et al., 2017), so classified information about the toxicity and safety of this valuable herbal medicine is needed.

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In the current chapter, we categorize the toxicity and safety of saffron, crocin, crocetin, picrocrocin, and safranal into two parts: in the first part, basic data about the toxicity of these agents in animal models are discussed. In the second part, we focus on the safety and adverse effects of saffron, which havev been studied in clinical trials.

34.2 Experimental data on the safety and toxicity of saffron and its bioactive ingredients in animal models

34.2.1 Acute toxicity

In acute toxicity tests, determination of the LD_{50} value (the amount of the substance required to kill 50% of the test population) is the main goal. A LD_{50} value can be affected by route of administration, strain, age, sex, type of feed, and weight of the animals (Eaton and Gilbert, 2015). Based on the LD_{50} values in acute toxicity tests, the chemical agents can be classified with different toxicity levels: supertoxic (<1 mg kg^{-1}), extremely toxic (<5 mg kg^{-1}), highly toxic (1−50 mg kg^{-1}), moderately toxic (50−500 mg kg^{-1}), slightly toxic (500−5000 mg kg^{-1}), practically nontoxic (5000−15,000 mg kg^{-1}), and relatively harmless ($>15{,}000$ mg kg^{-1}).

In this section we considered the LD_{50} of saffron, crocin, and safranal to evaluate toxicity. Different studies have reported saffron LD_{50} values:

1. Following oral administration of aqueous extract of saffron in BALB/c mice, the LD_{50} value was 4120 ± 556 mg kg^{-1} (Bahmani et al., 2014).
2. Intraperitoneal (IP) exposure of saffron stigma extract in mice resulted in an LD_{50} value of 1.6 g kg^{-1} (Karimi et al., 2004).
3. Following IP administration of saffron petal extract in mice, LD_{50} value 6 g kg^{-1} was found (Karimi et al., 2004).
4. Oral administration of ethanolic saffron extract in mice up to 5 g kg^{-1} did not produce any detectable acute toxic effects or deaths (Ramadan et al., 2012).

According to classification of chemicals based on LD_{50} values, it can be concluded that saffron extract is slightly toxic and practically nontoxic in acute exposure.

Administration of crocin (0.5, 1, 1.5, 2, and 3 g kg^{-1}, IP or orally 3 g kg^{-1}) did not show any mortality after 24 and 48 hours of treatment in mice. Regarding the tolerated dose of 3 g kg^{-1} of crocin (orally and IP administration), it has been found to be practically low toxic in acute ingestion or IP treatment (Hosseinzadeh et al., 2010).

The acute toxicity of safranal was evaluated in rat and mice within 2 days after IP or oral administration. The LD_{50} values of safranal were 1.48 mL kg^{-1} in male mice, 1.88 mL kg^{-1} in female mice, and 1.50 mL kg^{-1} in male rats following IP exposure, while oral LD_{50} values were 21.42 mL kg^{-1} in male mice, 11.42 mL kg^{-1} in female mice, and 5.53 mL kg^{-1} in male rats. According to this study, safranal was low toxic in acute IP exposure and practically nontoxic in acute oral administration in both mice and rats. Differences between oral and IP LD_{50} values in part can be due to the effects of first-pass metabolism and low absorption following oral treatment (Hosseinzadeh et al., 2013).

34.2.2 Subacute toxicity

In subacute toxicity tests, the toxicity of a chemical agent after repeated administration is studied. Biochemical parameters and histopathological findings are evaluated after either 14 or 28 days of exposure. The obtained data is used for dose selection in prolonged studies (Eaton and Gilbert, 2015).

Daily IP administration of aqueous extract of stigma (0.16, 0.32, and 0.48 g kg^{-1}) and petal (1.2, 2.4, and 3.6 g kg^{-1}) for 2 weeks markedly reduced RBC (red blood cell), HCT (hematocrit), Hb (hemoglobin), and induced anemia in rat. The aqueous extract of petal in 2.4 and 3.6 g kg^{-1} doses elevated the level of ALT (alanine aminotransferase), AST (aspartate aminotransferase), and LDH (lactate dehydrogenase) enzymes. Also, liver and lung injuries were observed following treatment by stigma extract (Karimi et al., 2004).

Repeated IP administration of ethanolic extract of saffron (0.35, 0.7, and 1.05 g kg^{-1}) for 2 weeks to rats reduced RBC, HCT, and Hb and increased WBC (white blood cell) counts and the levels of ALT and AST enzymes. Additionally, the level of serum uric acid, urea, and creatinine were significantly elevated. Based on the pathological studies, the ethanolic extract of saffron was found to induce mild-to-severe renal and hepatic injuries (Mohajeri et al., 2007).

In another study, oral administration of saffron at a dose of 200 mg kg^{-1} for 28 days could markedly decrease spermatogenesis index in rats (Khayatnouri et al., 2011).

Intraperitoneal administration of aqueous extract of saffron (200 mg kg^{-1}) three times per week for 4 weeks to rats increased the level of ALT, uric acid, and WBC (Hariri et al., 2018).

Saffron petal ethanolic extract (0, 75, 150, 225, and 450 mg kg^{-1}) was injected intraperitoneally to rats for 14 days. No significant changes in hematological parameters and spleen histology were observed. The level of IgG at dose of 75 mg kg^{-1} was significantly elevated in comparison to other groups (Babaei et al., 2014).

Overall, it can be concluded that anemia, liver, lung, and renal injuries are the main findings in subacute toxicity of saffron, especially in high doses.

In order to evaluate the hepatotoxicity of crocin in rat, different doses of this carotenoid (50, 100, and 200 mg kg^{-1}) were administrated IP once a week for 4 weeks. At the end of treatment, antioxidant enzymes including catalase, superoxide dismutase, and glutathione peroxidase (GPx) and serum biomarkers such as AST, ALP, ALT, uric acid, urea, and creatinine were measured. In addition, induction of oxidative stress was evaluated through measurement of protein carbonyl and malondialdehyde levels and glutathione content. No significant changes in serum parameters and oxidative stress biomarkers were observed. Also, histopathological analysis showed that crocin at these doses could not induce liver injury. The activity of GPx was reduced at higher dose of crocin (200 mg kg^{-1}) (Taheri et al., 2014). In another study, rats were treated with crocin (15, 45, 90, and 180 mg kg^{-1}, IP) for 21 days. Biochemical, hematological, and histopathological parameters as well as changes in weight and amount of food intake were measured in this study. According to the results, weight loss, reduction in food intake, decline in platelets and creatinine levels, elevation of LDL levels, reduction in alveolar size in lungs, myosin light chain atrophy, and decrease in size of cell nuclei in heart were the main findings following exposure to crocin (180 mg kg^{-1}) (Hosseinzadeh et al., 2010).

Administration of crocin (50 mg kg^{-1}, IP) for 4 weeks to rats did not induce cardiotoxicity, which was evaluated through oxidative stress assay, histopathological examination, and apoptosis markers analysis (Razavi et al., 2013a). Subacute administration of crocin (20 mg kg^{-1}) did not induce hepatotoxicity in rats. The normal level of liver biomarkers including ALT and AST, normal histopathological pattern of liver, no significant alteration in the level of mitogen-activated protein kinase (MAPK) pathway proteins, normal content of GSH and of 8-iso prostaglandine $_{F2a}$ confirmed crocin at this dose and duration of exposure could not induce liver injury (Vahdati-Hassani et al., 2017). Regarding the obtained results, it can be concluded that subacute treatment with crocin at these doses does not induce significant organ toxicity.

The subacute oral administration of safranal (0.1, 0.25, or 0.5 mL kg^{-1} daily for 21 days) markedly reduced RBC, Hb, HCT, and platelets and did not show any significant alteration in some biochemical markers including total bilirubin, serum glucose, albumin, serum creatinine, ALT, AST, CPK (creatine phosphokinase), and total bilirubin in rats. The levels of total cholesterol, triglyceride, and ALP were significantly reduced with 0.5 and 0.25 mL kg^{-1} doses of safranal. Also, significant elevation in the levels of LDH and serum urea nitrogen (BUN) was observed at higher dose of safranal. Safranal (0.5 mL kg^{-1}) induced noticeable abnormalities in lung and kidneys. The main findings were edema and cytolysis in kidney, progressed emphysema, and lymphocyte infiltration in lungs (Hosseinzadeh et al., 2013).

Toxic effects of safranal (0.1, 0.5, and 1 mL kg^{-1}, IP for 21 days) on humoral and cellular immune system of mice have been investigated. The results showed that safranal at studied doses and duration of exposure does not have immunotoxic properties (Riahi-Zanjani et al., 2015).

Overall it seems that subacute exposure to safranal at high doses induces toxicity.

34.2.3 Subchronic and chronic toxicity

A subchronic toxicity test will determine the toxic effects following repeated administration of a substance during 30–90 days. In this type of study, mortality, body weight, food/water consumption, hematology, urinalysis, organ weights, and histopathology are measured.

Generally, NOAEL (NO observed adverse effect level) and LOAEL (lowest observed adverse effect level) are determined during this test. The obtained data of subchronic tests are used basically for dose selection in chronic studies (Eaton and Gilbert, 2015).

Chronic toxicity tests are designed to evaluate the toxic effects as a result of more than 3 months of exposure. The duration of exposure is somewhat dependent on the intended period of exposure in humans. The purpose of this type of study is the assessment of physiological, pathological, pharmacokinetic, and carcinogenicity aspects of the substance (Eaton and Gilbert, 2015).

In order to determine the subchronic effects of saffron, the ethanolic extract (500 mg kg^{-1}, orally) was administrated to rats for 8 weeks. According to the results, saffron did not affect the serum activity of ALT and AST or the serum

levels of creatinine and urine. Other parameters including food consumption, food conversion ratio, and body weight gain were also not changed. But relative weights of kidneys and liver significantly increased in treated rats (Ramadan et al., 2012).

Following oral administration of aqueous saffron extract (120 mg kg^{-1}) for 105 days, no significant changes in metabolic parameters, organ weight, and levels of ALT and AST were observed (Lahmass et al., 2017).

There is less information about subchronic and chronic toxic effects of saffron and its main constituents, so the design of studies aimed at evaluating such effects are needed. Although according to the mentioned reports, no significant toxicity was observed at tested doses in subchronic exposure to saffron.

34.2.4 Developmental toxicity

Developmental toxicology explains any structural malformation, functional abnormality, and growth retardation caused by toxic chemicals or physical factors. It is the study of adverse effects on the development of the organism following exposure to toxic agents in different periods including before conception, during prenatal development, or postnatally until puberty (Carney et al., 2011; Eaton and Gilbert, 2015).

Due to the widespread use of herbal products, it is critical to understand how herbal medicines may affect embryonic development.

Different studies have been conducted to investigate the effects of saffron and its main constituents on fetal development. In order to investigate the teratogenic effects of saffron, pregnant BALB/c mice received different doses of aqueous saffron extract (0.2%, 0.4%, and 0.8%) in whole gestational period and third trimester throughout the gestational period. The results showed that placental weight and diameter, body and tail length, mean fetal weight, and biparietal diameter significantly decreased in animals treated with a higher dose of solution and mostly following exposure during the whole gestational period. Also, the mean number of resorbed and dead fetuses increased in a dose-dependent manner (Zeynali et al., 2009). Administration of saffron in the first or second trimester of the gestational period in mice elevated the mean number of resorbed and dead fetuses. The maximum resorbed fetuses were recorded in animals receiving 0.8% saffron solution during the first trimester while the maximum dead fetuses were observed in animals receiving higher doses of saffron during the second trimester (Hosseini et al., 2009).

In another study, saffron (2.5 and 100 mg kg^{-1}) was administrated to pregnant mice for 5 days during the first and second weeks of gestation and for 4 days during the third week of gestation. Embryonic toxicity and congenital malformations were evaluated on days 14 and 18 of gestation and day 1 neonates. Both doses of saffron led to significant reduction in embryonic growth parameters. Congenital malformations were observed in treated embryos and neonates including subcutaneous bleeding and head malformations (Al-Qudsi and Ayedh, 2012).

Oral administration of saffron (50, 250, and 1000 mg kg^{-1}) on day 5 until day 19 of gestation in Wistar rat did not affect maternal body weight gains, uterine weight, food intake, corpora lutea, and implantation counts, pre- and postimplantation loss, litter size, weight, and length. Also, soft tissue abnormalities and skeletal malformation were not observed (Edamula et al., 2014).

In order to determine postnatal developmental toxicity of saffron, pregnant Wistar rats were treated with saffron (50, 250, and 1000 mg kg^{-1}, orally) from implantation (day 5 postcoitus) through lactation up to lactation day (LD) 20. Results showed that maternal/lactation body weight gains, food intake, and fertility were unaffected. Also, saffron at tested doses did not exhibit any adverse effects on the mean number of pups born and weight of male and female pups and pup survivability. It can be concluded that saffron did not cause maternal toxicity or toxicity on the developing fetus/pups (Edamula et al., 2014).

The embryonic toxicity of the main constituents of saffron was investigated following IP administration of crocin (200 and 600 mg kg^{-1}) or safranal (0.075 and 0.225 mL kg^{-1}) on gestational days 6−15. The length and weight of fetuses markedly reduced in animals receiving crocin or safranal. Additionally, minor skeletal abnormalities, growth retardation, mandible, and calvaria malformations were the main findings in these groups (Moallem et al., 2013).

In another study, the effects of crocetin (10, 25, 50, 100, and 200 μM) on *Xenopus* embryos were evaluated and compared with teratogenic effects of all-trans retinoic acid (ATRA). Neither crocetin nor ATRA exhibited embryolethal effects at the tested doses. Head-to-tail length and eye diameters reduced in all doses of crocetin while cement gland length decreased in higher doses. Crocetin appears to be 236 times less potent at decreasing the length of frog embryo, 371 times less potent at reducing eye diameter, and 397 times less potent at decreasing cement gland size in comparison to ATRA. Clearly, crocetin showed much fewer teratogenic effects than ATRA in all tested parameters (Martin et al., 2002).

As can be seen, saffron and its main constituents exhibited teratogenic effects particularly at high doses.

34.2.5 Mutagenicity and genotoxicity

Genotoxicity testing is generally performed to determine the destructive effects of substances on the genetic material of cells (DNA, RNA). Different in vivo and in vitro tests including genotoxicity bacterial reverse mutation test (Ames test), mammalian bone marrow chromosome aberration test, micronucleus test, mouse heritable translocation assay, and in vitro sister chromatid exchange assay in mammalian cells have been used to evaluate genotoxicity (Jena et al., 2002; Saks et al., 2017).

The antimutagenic and comutagenic effects of saffron and its main constituents were evaluated using the Ames/*Salmonella* test system and two well-known mutagens BP (benzo[a]-pyrene) and 2AA (2-amino-antracene). Saffron extract (100−1500 μg per plate) did not induce genotoxic effects using the *Salmonella typhimurium* tester strain TA98, both with and without S9 activation, but comutagenic effect of saffron on 2-AA-induced mutagenicity dose-dependently was detected. Crocin and picrocrocin at different concentrations (100−400 nM) per plate exhibited no comutagenic effects, while safranal at these doses markedly induced the comugagenic property on 2-AA-induced mutagenicity. It appears that the comutagenic effect of saffron is related to safranal (Abdullaev et al., 2003).

Crocetin did not show genotoxic effect in V79 cells, which was determined using the Ames test, rec-assay, and sister chromatid exchange test (Ozaki et al., 2002).

Single IP administration of safranal (5 mL kg^{-1}) to NMRI mice did not induce DNA migration in liver, kidney, and spleen organs, as determined by comet assay (Hosseinzadeh and Sadeghnia, 2007).

Administrations of saffron extract (50, 100, and 200 mg kg^{-1} IP, three times per week) (Hariri et al., 2018) or crocin (50, 100, and 200 mg kg^{-1}, IP, three times per week) did not elevate micronucleus indices (Hariri et al., 2011), while treatment of rats with safranal (0.1 mL kg^{-1}, IP, three times per week) significantly increased micronucleus index as a marker of genotoxicity (Hariri et al., 2011).

It can be concluded that safranal at high doses can induce genotoxicity.

Experimental studies related to the safety and toxicity of saffron, crocin, and safranal are summarized in Tables 34.1−34.3, respectively.

TABLE 34.1 Experimental studies related to safety and toxicity of saffron.

Toxicity	Route, dose, and duration of exposure	Animal model	Results	References
Acute	Oral administration of aqueous extract of saffron	BALB/c mice	LD_{50}: 4120 ± 556 mg kg^{-1}	Bahmani et al. (2014)
	IP exposure of saffron stigma extract	Male Wistar rat	LD_{50}: 1.6 g kg^{-1}	Karimi et al. (2004)
	IP exposure of saffron petal extract	Male Wistar rat	LD_{50}: 6 g kg^{-1}	Karimi et al. (2004)
	Oral administration of ethanolic saffron extract	Male mice	No detectable acute toxic effects or deaths up to 5 g kg^{-1}	Ramadan et al. (2012)
Subacute	IP, aqueous extract of stigma (0.16, 0.32, 0.48 g kg^{-1}), 2 weeks	Male Wistar rat	Reduction of RBC, HCT, Hb, and induction anemia in rat	Karimi et al. (2004)
			Liver and lung injuries	
	IP, aqueous extract of petal (1.2, 2.4, 3.6 g kg^{-1}) g kg^{-1}, 2 weeks	Male Wistar rat	Reduction of RBC, HCT, Hb, and induction anemia in rat	Karimi et al. (2004)
			Elevation of ALT, AST, and LDH levels in higher doses	
	IP, ethanolic extract (0.35, 0.7, 1.05 g kg^{-1}) 2 weeks to rats	Male Wistar rat	Reduction of RBC, HCT, Hb	Mohajeri et al. (2007)
			Elevation of WBC, ALT, AST, urea, uric acid, and creatinine	
			Mild-to-severe hepatic and renal injuries	
	Oral, saffron 200 mg kg^{-1} for 28 days	Male rat	Reduction of spermatogenesis index	Khayatnouri et al. (2011)

(*Continued*)

TABLE 34.1 (Continued)

Toxicity	Route, dose, and duration of exposure	Animal model	Results	References
	IP, aqueous extract of saffron (200 mg kg^{-1}), three times per week for 4 weeks	Male Wistar rat	Elevation of WBC, ALT, and uric acid	Hariri et al. (2018)
	IP, Saffron petal ethanolic extract (0, 75, 150, 225, and 450 mg kg^{-1}) for 14 days	Male Wistar rat	No significant changes in hematological parameters and spleen histology were observed	Babaei et al. (2014)
			Elevation the level of IgG at dose of 75 mg kg^{-1}	
Subchronic	Oral, ethanolic extract of saffron (500 mg kg^{-1}), for 8 weeks	Male sprague dawley rat	No significant changes in AST, ALT, creatinine, urine, food consumption, food conversion ratio, and body weight gain	Ramadan et al. (2012)
			Relative weights of kidneys and liver significantly increased	
	Oral, aqueous extract of saffron (120 mg kg^{-1}), for 105 days	Male Wistar rat	No significant changes in metabolic parameters, organ weight, ALT, and AST levels	Lahmass et al. (2017)
Developmental toxicity	Oral, aqueous saffron extract (0.2%, 0.4%, 0.8%) 1st or	Pregnant BALB/c mice	Reduction of placental weight and diameter, mean fetal weight, body and tail length, and biparietal diameter	Hosseini et al. (2009)
	2nd trimesters throughout the gestational period		Elevation of the mean number of resorbed and dead fetuses	
	Oral, aqueous saffron extract (0.2%, 0.4%, 0.8%) in whole gestational period and 3rd trimester throughout the gestational period	Pregnant BALB/c mice	Reduction of placental weight and diameter, mean fetal weight, body and tail length, and biparietal diameter	Zeynali et al. (2009)
			Elevation of the mean number of resorbed and dead fetuses	
	Oral, aqueous saffron extract (2.5, 100 mg kg^{-1}) to for 5 days during the 1st and 2nd weeks of gestation and for 4 days during the 3rd week of gestation	Pregnant mice	Reduction of embryonic growth parameters subcutaneous bleeding, and head malformations	Al-Qudsi and Ayedh (2012)
	Oral, saffron (50, 250, 1000 mg kg^{-1}), during day 5 until day 19 of gestation	Pregnant Wistar rat	No changes in maternal body weight gains, food intake, uterine weight, corpora lutea, implantation counts, pre- and postimplantation loss, litter size, weight, and length. No soft tissue abnormalities and skeletal malformation.	Edamula et al. (2014)
	Oral, saffron (50, 250, 1000 mg kg^{-1}) from implantation (day 5 postcoitus) through lactation up to LD 20	Pregnant Wistar rats	No changes in maternal/lactation body weight gains, food intake, no adverse effects on the mean number of pups born and weight of male and female pups and pup survivability	Edamula et al. (2014)
Genotoxicity	Oral, aqueous saffron extract (50, 100, 200 mg kg^{-1}) three times per week for 4 weeks	Male Wistar rat	No changes in micronucleus index	Hariri et al. (2018)
	Saffron extract (100–1500 μg $plate^{-1}$)	Ames/ *Salmonella typhimurium* test	No genotoxic effect	Abdullaev et al. (2003)
			Comutagenic effect on 2-amino-antracene-induced mutagenicity	

ALT, Alanine aminotransferase; *AST*, aspartate aminotransferase; *Hb*, hemoglobin; *HCT*, hematocrit; *IP*, intraperitoneal; *LDH*, lactate dehydrogenase; *RBC*, red blood cell; *WBC*, white blood cell.

TABLE 34.2 Experimental studies related to safety and toxicity of crocin.

Toxicity	Dose, route, and duration of exposure	Animal model	Results	References
Acute	IP (0.5, 1, 1.5, 2, 2.5, 3 g kg^{-1})	Male razi mice	Tolerated dose: 3 g kg^{-1}	Hosseinzadeh et al. (2010)
	Orally (3 g kg^{-1})			
Subacute	IP (50, 100 and 200 mg kg^{-1}) once a week for 4 weeks	Female Wistar albino	No significant changes in serum parameters and oxidative stress biomarkers	Taheri et al. (2014)
			No liver injury	
			Reduction of GPx activity at 200 mg kg^{-1}	
	IP (15, 45, 90, 180 mg kg^{-1}) for 21 days	Male Wistar rat	Weight loss, reduction in food intake, decline in, platelets and creatinin levels, elevation of LDL level, reduction in alveolar size in lungs, myosin light chain atrophy, decrease in size of cells nuclei in heart were the main findings following exposure to crocin (180 mg kg^{-1})	Hosseinzadeh et al. (2010)
	IP (50 mg kg^{-1}) for 4 weeks	Male Wistar rat	No changes in oxidative stress, histophatological, and apoptotic markers in heart	Razavi et al. (2013a)
	IP (20 mg kg^{-1}) for 4 weeks	Male Wistar rat	No changes in oxidative stress, biochemical and MAPK proteins in liver	Vahdati-Hassani et al. (2017)
Developmental toxicity	IP (200, 600 mg kg^{-1}) on gestational days 6–15.	Pregnant mice	Reduction of length and weight of fetuses	Moallem et al. (2013)
			Minor skeletal abnormalities, growth retardation, mandible, and calvaria malformations	
Genotoxicity	100–400 nM $plate^{-1}$	Ames/ *Salmonella typhimurium* test	No comutagenic effect on on 2-amino-antracene-induced mutagenicity	Abdullaev et al. (2003)

GPx, Glutathione peroxidase; *IP*, intraperitoneal; *LDL*, low-density lipoprotein; *MAPK*, mitogen-activated protein kinase.

34.3 Clinical studies on safe and toxic doses of saffron and its bioactive ingredients

Saffron has been utilized as a food additive without complications for several centuries. According to some studies, doses up to 1.5 g day^{-1} are believed to be safe and adverse effects are reported with doses more than 5 g day^{-1} with lethal dose of approximately 20 g. It has been reported that doses more than 10 g day^{-1} have been used for induction of abortion. Some adverse effects including vomiting, uterus bleeding, hematuria, bleeding of the gastrointestinal mucosa as well as vertigo and dizziness have been reported at this dose (Schmidt et al., 2007). The safety of saffron has been directly reported in few studies. For example, in healthy volunteers, crocin (20 mg day^{-1}) for 1 month did not show any clinically important adverse effects in comparison with placebo, although reductions in serum amylase, mixed WBCs, and PTT were observed (Mohamadpour et al., 2013). For evaluating the safety of saffron, a double-blind, placebo-controlled study with healthy adult volunteers was performed. Saffron tablets (400 mg day^{-1}, for 1 week) did not show any significant adverse effects on blood pressure except for reducing standing systolic blood pressure and mean arterial pressures (Modaghegh et al., 2008). In addition, some hematological parameters such as RBCs, hemoglobin, hematocrit, and platelets were reduced slightly by saffron tablets (200 and 400 mg, for 1 week). Saffron also elevated sodium, blood urea nitrogen, and creatinine. These alterations were in normal ranges and were not clinically important (Modaghegh et al., 2008).

TABLE 34.3 Experimental studies related to safety and toxicity of safranal.

Toxicity	Dose, route, and duration of exposure	Animal model	Results	References
Acute	IP	Male BALB/c mice	LD_{50}: 1.48 mL kg^{-1}	Hosseinzadeh et al. (2013)
	IP	Female BALB/c mice	LD_{50}: 1.88 mL kg^{-1}	Hosseinzadeh et al. (2013)
	IP	Male Wistar rats	LD_{50}: 1.50 mL kg^{-1}	Hosseinzadeh et al. (2013)
	Oral	Male BALB/c mice	LD_{50}: 21.42 mL kg^{-1}	Hosseinzadeh et al. (2013)
	Oral	Female BALB/c mice	LD_{50}: 11.42 mL kg^{-1}	Hosseinzadeh et al. (2013)
	Oral	Male Wistar rats	LD_{50}: 5.53 mL kg^{-1}	Hosseinzadeh et al. (2013)
Subacute	Oral (0.1, 0.25, or 0.5 mL kg^{-1}) daily for 21	Male Wistar rats	Reduction of RBC, Hb, HCT, and platelets	Hosseinzadeh et al. (2013)
			Reduction of total cholesterol, triglyceride, and ALP in 0.5 and 0.25 mL kg^{-1} doses	
			Elevation of the level of LDH and BUN, kidney and lung abnormalities at 0.5 mL kg^{-1}	
	IP (0.1, 0.5, 1 mL kg^{-1}) for 21 days	Male BALB/ cimice	No immunotoxic effects	Riahi-Zanjani et al. (2015)
Developmental toxicity	IP, (0.075 and 0.225 mL kg^{-1}) on gestational days 6–15	Pregnant mice	Reduction of length and weight of fetuses	Moallem et al. (2013)
			Minor skeletal abnormalities, growth retardation, mandible, and calvaria malformations	
Genotoxicity	100–400 nM $plate^{-1}$	Ames/ *Salmonella typhimurium* test	Comutagenic effect on on 2-amino-antracene-induced mutagenicity	Abdullaev et al. (2003)

ALP, Alkaline phosphatase; *BUN*, blood urea nitrogen; *Hb*, hemoglobin; *HCT*, hematocrit; *LDH*, lactate dehydrogenase; *RBC*, red blood cell; *WBC*, white blood cell.

In another clinical trial, the safety of saffron and crocin in patients with schizophrenia was evaluated. Toxic effects on thyroid, liver, kidney, and hematologic systems were not observed by saffron and crocin (15 mg twice daily) (Mousavi et al., 2015). The results of this study provides evidence for the safety of saffron.

Consumption of saffron capsules (250 mg) in the active phase of the first stage of labor reduced the intensity of pain from 97.4 ± 2.9 in placebo group to 85.9 ± 8.4 in treated females, and did not show toxicity in infants and mothers (Ahmadi et al., 2014). Moreover, administration of three 250 mg saffron pills in 24 hours to women with a gestational age of 39–41 weeks increased the readiness of the cervix in term pregnancies without any reported adverse effects (Sadi et al., 2016). In another double-blind, placebo-controlled study, 1 week treatment with saffron tablets (200 and 400 mg day^{-1}) did not show any adverse effects on the coagulant and anticoagulant system (Ayatollahi et al., 2014).

According to a meta-analysis of randomized controlled trials to evaluate the efficacy and safety of saffron in the treatment of major depressive disorder (MDD) compared to placebo and synthetic antidepressants, saffron was

considered as a safe drug without serious adverse events (Yang et al., 2018). In these studies, the stigma and petal of saffron [capsules of 30 mg day^{-1} (BID)] for 4, 6, and 8 weeks were effective in the treatment of mild-to-moderate depression without any important adverse effects (Akhondzadeh-Basti et al., 2007; Akhondzadeh et al., 2005, 2004; Ghajar et al., 2017; Moshiri et al., 2006; Noorbala et al., 2005; Shahmansouri et al., 2014).

In addition, the efficacy and safety of saffron in the treatment of patients with mild-to-moderate Alzheimer's disease were evaluated. Patients randomly received saffron capsules (15 mg twice per day) for 22 (Akhondzadeh et al., 2010b) or 16 (Akhondzadeh et al., 2010a) weeks. The effect of saffron was compared to donepezil (Akhondzadeh et al., 2010b) or placebo (Akhondzadeh et al., 2010a). According to the results, saffron at this dose acted similar to donepezil in the treatment of mild-to-moderate Alzheimer's disease after 22 weeks and considerably improved cognitive function more than placebo after 16 weeks. The frequency of adverse effects was not significant between saffron and placebo or donepezil groups. Only vomiting occurred significantly more frequently in the donepezil group (Akhondzadeh et al., 2010a,b).

In a randomized, double-blind, placebo controlled clinical trial study, the safety of coadministration of saffron and SSRI on sexual dysfunction in patients with MDD was evaluated. Saffron (15 mg twice daily) for 4 weeks did not change significantly laboratory parameters such as liver and kidney function tests, blood cell counts, and coagulation tests (Mansoori et al., 2011). Moreover, in another clinical study conducted on patients with anxiety and depression, a 50 mg saffron capsule (*Crocus sativus* L. stigma) twice daily for 12 weeks showed a significant impact on the treatment of anxiety and depression disorder without any side effects (Mazidi et al., 2016). The safety of saffron extract (Affron, 14 mg BID) which was given to teenagers aged 12−16 years with mild-to-moderate anxiety or depressive symptoms for 8 weeks was evaluated. Saffron improved anxiety and depressive symptoms. Between placebo and saffron extract groups no significant adverse effects were observed. Only increased frequency of headaches in the placebo group compared to saffron group was observed (Lopresti et al., 2018). Furthermore, Affron (28 mg day^{-1}) for 4 weeks increased mood and decreased anxiety in healthy adults without side effects (Kell et al., 2017).

To evaluate the safety of long-term or excessive crocetin consumption in healthy adult volunteers, physical examination, blood biochemistry, hematology, and urine were performed. The results showed that taking a crocetin capsule (7.5 mg per capsule) once per day for 12 consecutive weeks or 37.5 mg day^{-1} for 4 consecutive weeks was safe (Yamashita et al., 2018a,b). To evaluate the effect of crocin on diabetic maculopathy, a double-blind, placebo controlled, phase 2 randomized clinical trial was done. The results showed that administration of crocin 15 mg day^{-1} for 3 months could significantly decrease HbA1c and central macular thickness and improve best-corrected visual acuity compared to the placebo group. Some side effects including increased appetite, subconjunctival hemorrhage, swelling, redness, and burning of the eyes associated with pain in some cases were reported. No statistical significant difference was observed between groups for the side effects discussed earlier (Sepahi et al., 2018).

Additionally, the safety and efficacy of saffron (30 mg day^{-1}, BID) for 10 weeks in the treatment of mild-to-moderate obsessive-compulsive disorder were proven in another clinical study. Saffron was as effective as fluvoxamine (100 mg day^{-1}), and the frequency of adverse effects was not significantly different between the two groups (Esalatmanesh et al., 2017).

Daily consumption of saffron (30 mg) reduced the severity of mild-to-moderate postpartum depressive disorder with no significant adverse effects on breastfeeding mothers or infants. Some adverse effects such gastrointestinal disorders, lack of sleep or oversleeping, low breast milk, and bleeding gums were observed in breastfeeding mothers that were not significantly important (Tabeshpour et al., 2017). Another double-blind, randomized clinical trial showed that consumption of saffron capsules (15 mg capsule) twice daily for 6 weeks is a safe alternative medication for reducing symptoms of postpartum depression. For patients taking fluoxetine (20 mg capsule) more headache, dry mouth, daytime drowsiness, constipation, and sweating side effects than the saffron group were reported that were not significantly important (Kashani et al., 2017).

To evaluate the effects of saffron on the immune system, one 100 mg saffron tablet daily for 6 weeks was administrated to healthy men. Saffron elevated the IgG level and reduced the IgM level, the percentage of basophils and the platelets count, but increased the monocytes percentage after 3 weeks. After 6 weeks, these parameters returned to baseline levels. No adverse effects were reported (Kianbakht and Ghazavi, 2011).

In summary, the common effective doses of saffron used in clinical practice (30−50 mg day^{-1}) were noticeably lower than toxic doses (>5 g day^{-1}), so saffron has a wide therapeutic index (Table 34.4).

TABLE 34.4 Clinical studies related to safety of saffron.

Study design	Dose/ constituents	Duration of exposure	Adverse effects	References
Double-blind, placebo-controlled study in the healthy adult volunteers	Crocin (20 mg day^{-1})	1 month	↓ Serum amylase, mixed WBCs, PTT	Mohamadpour et al. (2013)
Double-blind, placebo-controlled study in the healthy adult volunteers	Saffron tablets (200 and 400 mg day^{-1})	1 week	↓ Standing SBP, MAP	Modaghegh et al. (2008)
			↓ RBCs, Hb, HCT, platelets	
			↑ Na, BUN, Cr	
Double-blind, placebo-controlled study was performed on patients with schizophrenia	Saffron aqueous extract and crocin (15 mg day^{-1}, BID)	12 weeks	No toxic effects on thyroid, liver, kidney, and hematologic systems	Mousavi et al. (2015)
Double-blind, placebo-controlled trial	Saffron capsule (250 mg)	Active phase of the first stage of labor	No significant adverse effect	Ahmadi et al. (2014)
Double-blind, placebo-controlled trial	Saffron (250 mg TID^{-1})	24 hours	No significant adverse effect	Sadi et al. (2016)
Double-blind, placebo-controlled study	Saffron tablet (200 and 400 mg day^{-1})	1 week	No adverse effect on coagulant and anticoagulant system	Ayatollahi et al. (2014)
Double-blind, randomized and placebo-controlled trial	Saffron capsule (30 mg day^{-1}, BID)	6 weeks	No significant differences in saffron and placebo groups in terms of the side effects	Akhondzadeh et al. (2005)
Double-blind, placebo-controlled and randomized trial	Saffron petal capsule (30 mg day^{-1}, BID)	6 weeks	No significant differences in saffron and placebo groups in terms of the side effects	Moshiri et al. (2006)
Randomized double-blind parallel-group study on depressed patients	Saffron capsule (30 mg day^{-1}, BID)	6 weeks	No significant differences between saffron and fluoxetine groups in the frequency of adverse events	Shahmansouri et al. (2014)
Double-blind randomized trial on depressed outpatients	Saffron capsule (30 mg day^{-1}, BID)	8 weeks	No significant differences between saffron and fluoxetine groups in the frequency of adverse events	Akhondzadeh-Basti et al. (2007)
Double-blind, randomized trial on depressed patients	Hydroalcoholic extract of saffron capsule (30 mg day^{-1}, BID)	6 weeks	No significant differences between saffron and fluoxetine groups in the frequency of adverse events	Noorbala et al. (2005)
Double-blind, randomized trial on depressed patients	Saffron capsule (30 mg day^{-1}, TDS)	6 weeks	Dry mouth and sedation were observed in imipramine group significantly more frequently than the saffron group	Akhondzadeh et al. (2004)
Double-blind, randomized trial on depressed patients	Saffron capsule (30 mg day^{-1}, BID)	6 weeks	No significant differences between saffron and citalopram groups in the frequency of adverse events	Ghajar et al. (2017)
Double-blind, randomized trial on patients with AD	Saffron capsule (30 mg day^{-1}, BID)	22 weeks	Vomiting occurred significantly more frequently in the donepezil group than in the saffron group	Akhondzadeh et al. (2010b)
Double-blind, randomized trial on patients with AD	Saffron capsule (30 mg day^{-1}, BID)	16 weeks	No significant differences between saffron and placebo groups in the frequency of adverse events	Akhondzadeh et al. (2010a)

(*Continued*)

TABLE 34.4 (Continued)

Study design	Dose/ constituents	Duration of exposure	Adverse effects	References
Double-blind, placebo controlled clinical trial on depressed patients	Saffron capsule (30 mg day^{-1}, BID) and SSRI	4 weeks	No significant differences between saffron and placebo groups in the frequency of adverse events	Mansoori et al. (2011)
Double-blind, randomized, placebo controlled trial on patients with anxiety and depression	Saffron capsule (50 mg, BID)	12 weeks	Side effects were rare	Mazidi et al. (2016)
Randomized, double-blind, placebo-controlled trial on teenagers with anxiety or depressive symptoms	Saffron extract (Affron, 14 mg BID)	8 weeks	Headache occurred more frequently in the placebo group than in the saffron group	Lopresti et al. (2018)
Randomized, double-blind and placebo-controlled trial on healthy adults	Saffron extract (Affron, 14 mg BID)	4 weeks	No adverse effect	Kell et al. (2017)
Randomized, double-blind, placebo-controlled trial	Crocetin capsule (7.5 mg day^{-1})	12 weeks	No adverse effect	Yamashita et al. (2018b)
Randomized, double-blind, placebo-controlled trial	Crocetin capsule (37.5 mg day^{-1})	4 weeks	No adverse effect	Yamashita et al. (2018a)
Double-blind, placebo controlled, phase 2 randomized clinical trial on diabetic patients	Crocin (15 mg day^{-1})	3 months	No significant differences between crocin and placebo groups in the frequency of adverse events	Sepahi et al. (2018)
Double-blind, randomized trial on patients with obsessive-compulsive disorder	saffron (30 mg day^{-1}, BID)	10 weeks	No significant differences between saffron and fluvoxamine groups in the frequency of adverse events	Esalatmanesh et al. (2017)
Double-blind, randomized and placebo-controlled trial on women with postpartum depressive disorder	Saffron (30 mg day^{-1})		No significant differences between saffron and placebo groups in the frequency of adverse events	Tabeshpour et al. (2017)
Double-blind, randomized clinical trial on women with postpartum depressive disorder	Saffron (15 mg, BID)	6 weeks	No significant differences between saffron and fluoxetine groups in the frequency of adverse events	Kashani et al. (2017)
Randomized double-blind placebo-controlled clinical trial on healthy men	Saffron tablet (100 mg day^{-1})	3 weeks	↑ IgG level	Kianbakht and Ghazavi (2011)
			↓ IgM level	
			↓ % basophils	
			↓ Platelet counts	
			↑ % monocytes	

BUN, Blood urea nitrogen; *Cr*, creatinine; Hb, hemoglobin; *HCT*, hematocrit; *MAP*, mean arterial pressure; *PTT*, partial thromboplastin time; *RBC*, red blood cell; *SBP*, systolic blood pressure; *WBC*, white blood cell.

34.4 Conclusion

Different animal and clinical studies have been carried out to evaluate the toxicity and safety of saffron. In experimental studies, according to LD_{50} values, saffron, crocin, and safranal were found to be low or nontoxic agents. Exposure to high doses of saffron and its ingredients can induce organ and embryonic toxicity. Saffron at high doses may increase miscarriage rate in pregnant females, so using high doses of saffron during pregnancy should be avoided. According to some clinical reports saffron has a wide therapeutic index. Finally, as discussed in the chapter, the therapeutic doses of saffron in both clinical and experimental studies did not induce significant toxicity.

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