ACUTE AND SUB-ACUTE TOXICITY OF CROCIN, A CONSTITUENT OF *CROCUS SATIVUS* L. (SAFFRON), IN MICE AND RATS

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Summary

Acute and sub-acute toxicity of crocin, a constituent of *Crocus sativus*, were studied in mice and rats. The acute (up to 3 g, orally and intraperitoneally) and chronic (15, 45, 90 and 180 mg/kg, intraperitoneally) toxicity were evaluated for 2 and 21 days, respectively. In chronic toxicity, changes in weight and amount of food intake as well as biochemical, hematological and pathological tests were studied in rats after 21 days. High oral and intraperitoneal doses of crocin (3 g/kg) did not cause death within 2 days of study. A dose 180 g/kg of crocin in sub-acute study increased platelets and creatinin levels. The weight loss and a reduction in food intake were also observed with this concentration. The lower doses of the substance decreased albumin and ALP, and raised the LDL level dose-independently. A decline in alveolar size in lungs of 180 mg/kg crocin group was also observed. Moreover, in hearts of this group myosin light chain atrophy and a reduction in size of cells nuclei was seen. Despite the excessive tubular loss of crocin that made the urine orange-yellow, there was not a gross pathologic change in kidney. The results obtained in this study indicate that crocin at pharmacological doses did not exhibit marked damages to all the major organs of the body.

Keywords: Crocin, Saffron, *Crocus sativus*, Acute toxicity, Sub-acute toxicity

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Introduction

Crocus sativus (Iridaceae) L. stigma, commonly known as saffron, is cultivated mostly in Iran**.** The main pharmacologically active and important constituents of saffron are volatile agents (e.g. safranal), bitter principles (e.g. picrocrocin) and dye materials (e.g. crocetin and its glycosidic, crocin) (1).

Saffron extracts or its main constituents have shown different activities such as anticancer (2, 3), antidepressant (4, 5), anticonvulsant (6, 7, 8), memory enhancer (9, 10, 11), anxiolytic and hypnotic (12), attenuated cerebral ischemia (13) and reduced the extracellular hippocampal levels of glutamate and aspartate (14).

Crocin is a di-gentiobiose ester of crocetin and a water-soluble carotenoid of *C. sativus* that has attracted scientific attentions because of the various pharmacological effects such as hypotensive effect (15), antidepressant activity (4) genoprotective (16), improve sexual activity (17), antioxidant (18), inhibition of lipid peroxidation in renal (19), hippocampal (13) and muscle skeletal homogenates during ischemia-reperfusion-induced oxidative damage in rats (20).

Crocin is also reported to increase the blood flow in the retina and choroid and could be used in treatment of ischemic retinopathy and age-related macular degeneration. Moreover, crocin inhibits skin tumor growth (21), improves the learning behavior that has been impaired by ethanol (22) or hyoscine (11), and prevents the inhibitory effect of ethanol on long-term potentiation in rats (23).

In animal study, LD50 values of intraperitoneally injection of saffron stigma and petal extracts were 1.6 and 6 g/kg, respectively in mice. In sub-acute study, the aqueous extract of stigma decreased levels of hematocrit, hemoglobin and erythrocytes, but it did not induce any significant pathological effects on different organs (24). Information on toxicology and safety of saffron is not consistent. In some reports, saffron doses between 1.2 and 2 g showed nausea followed by vomiting, diarrhea and bleeding (25). Saffron tablets with high doses (200 and 400 mg/day) changed some hematological and biochemical parameters in healthy adult volunteers. However, these alterations were in normal ranges and they were not clinically important (26). As crocin, an active constituent of saffron showed a variety of interesting pharmacological activities, its acute and sub-acute toxicity were evaluated in mice and rats.

Material and Methods

Crocin extract and purification

Stigmas of *C. sativus* L. from Novin Saffron (collected from Ghaen, Khorasan province, Northeast of Iran) was obtained and analyzed in accordance to the ISO/TS 3632-2. Crocin was extracted and purified as defined by Hadizadeh and colleagues (27).

Animals

Male, Razi mice (weight 20–30 g) and Wistar rats (150-210 g) of a random-bred colony were obtained from the animal house of Mashhad University of Medical Sciences. Animals were housed in a room with a 12-hour light and dark cycle with a temperature of 21 ± 2°C and free access to water and food. All experiments on animals were performed according to Mashhad University of Medical Sciences, Ethical Committee Acts.

Acute toxicity study

Five mice were fed orally with single doses of 3 g and five groups (five mice in each group) were received crocin intraperitoneally at doses 0.5, 1, 1.5, 2, 2.5 and 3 g/kg. A 24 and 48-hour observation was performed to determine the mortality and toxicity signs.

Sub-acute toxicity study

Rats were divided into 5 groups, each of which contained 6 animals. Group 1 was the control group in which saline solution was administered (10 ml/kg) i.p.; groups 2-5 were given four concentration of crocin (15, 45, 90, 180 mg/kg) i.p. once a day during 21 days. During the experiment, changes of weight and amount of food intake of rats were recorded. On the last day, the rats were anesthetized using a mixture of xylazine (10 mg/kg) and ketamine (100 mg/kg), and blood was obtained from heart after an abdominal incision. The main organs including heart, lung, liver, kidney and spleen were then separated precisely and maintained in 10% formalin for further pathological tests. Cell-counter and auto-analyzer were used for hematology and biochemical tests, respectively. Paraffin blocks of principle organs were also prepared and after being sectioned by microtome, dyed with hematoxylin–eosin and the necessary assessments were made, based on pathological and histological tests**.**

Statistical analysis

Data is expressed as mean ± SEM. Statistical analysis was performed by using one-way ANOVA and then Tukey-Kramer post-test for multiple comparisons. The p-values less than 0.05 were considered statistically significant.

Results and Discussion

No mortality was seen with crocin (0.5, 1, 1.5, 2 and 3 g/kg, i.p. or orally 3 g/kg) after 24 and 48 hours of treatment. According to Loomis and Hayes (1996) classification (28), chemical substance with a LD_{50} within the range of 1–5 g/kg is considered as practically low-toxic. It seems in respect to tolerated dose of 3 g/kg of crocin (orally and intraperitoneally administration), this constituent of saffron should be regarded as practically low toxic in acute ingestion or i.p. treatment. There was not a significant difference in whole blood tests of experimental groups, but crocin at high dose (180 mg/kg) slightly increased platelet count (Plt × 10^3µl) ($p<0.05$) (Tables 1 and 2). Although platelet count in this group was lower than control group of other doses of crocin.

Previous studies showed that saffron decreased hemoglobin and hematocrit levels and total RBC count (24, 29). Comparing the experimental groups, there was a reduction in weight and food consumption only in the 180 mg/kg crocin group in comparison with the saline groups, while it does not indicate a significant change in other doses (Figures 1 and 2). Food consumption decreased in this group leading to poor weight gain in comparison with the saline group (Figures 3 and 4). It is possible the lower body weight in rats given crocin at high dose would be the lower food intake in this group of rats compared to the normal rat group. This suggests that crocin may cause a reduction in appetite, which leads to a reduced food consumption. Daily supplementation over 8 weeks with a food supplement, a patented extract of saffron stigma, reduced significantly the frequency of snacking events and a slight body weight loss in healthy women (30).

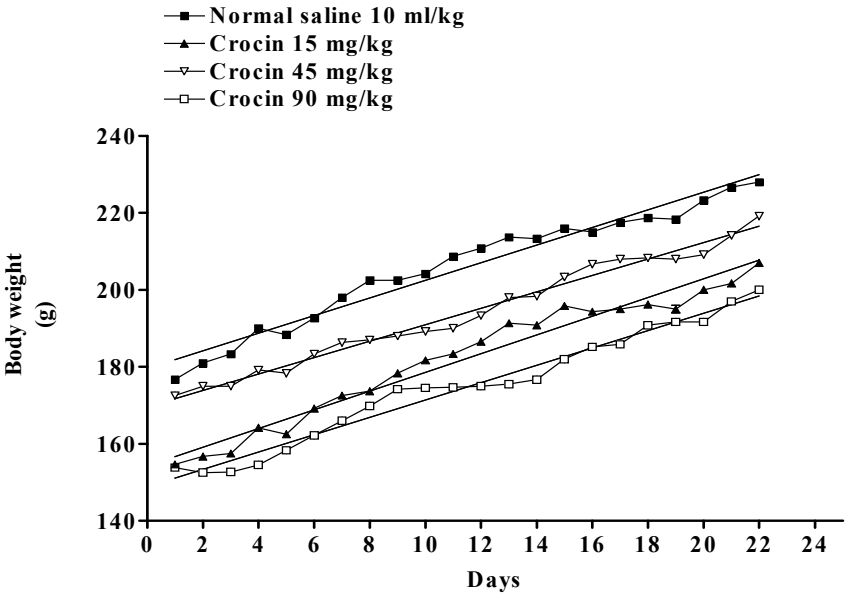

Figure 1. Effect of intraperitoneally administration of crocin on body weight. Crocin and control were administered intraperitoneally once a day during 21 days. The rate of weight gain in all groups was relatively the same as each other.

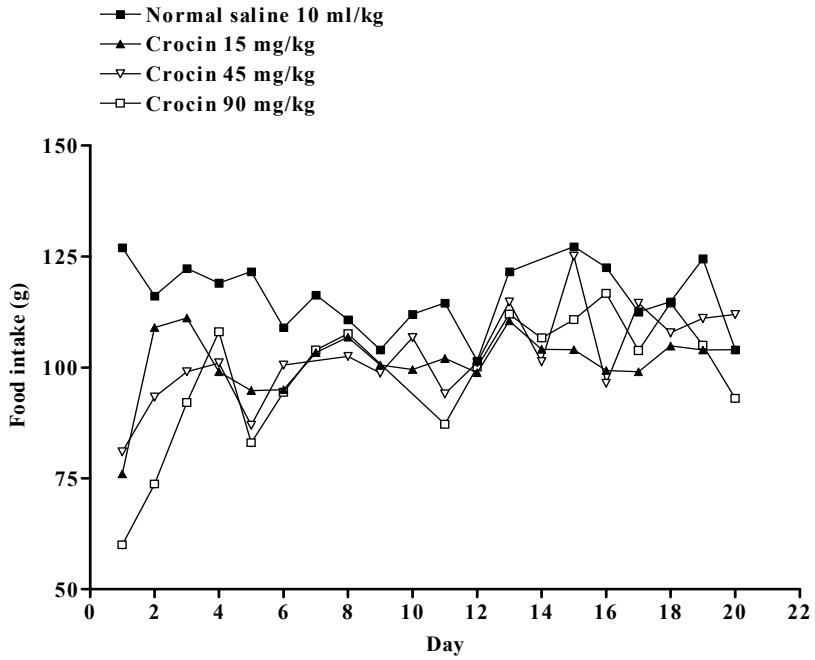

Figure 2. Effect of intraperitoneally administration of crocin on food intake. Crocin and control were administered intraperitoneally once a day during 21 days. There is a significant change in amount of food intake in the primary days, but as the weight of rats get closer the amount of food intake show more similarities in experimental groups indicating the therapeutic substance has no role.

Table 1- Effect of crocin on hematological parameters after 21 days treatment in doses from 15 to 90 mg/kg

Tests	Normal saline 10 ml/kg	Crocin 15 mg/kg	Crocin 45 mg/kg	Crocin 90 mg/kg
WBC($\times10^3\mu l$)	11.20 ± 0.65	10.53 ± 1.35	12.07 ± 2.37	8.43 ± 0.32
RBC($\times10^6\mu l$)	8.24 ± 0.18	8.29 ± 0.15	7.85 ± 0.17	8.23 ± 0.11
HGB(g/dl)	14.12 ± 0.27	14.02 ± 0.40	13.90 ± 0.37	14.30 ± 0.17
HCT (%)	42.66 ± 0.86	42.08 ±0.95	40.77 ± 0.96	44.03 ± 1.08
MCV(fL)	51.88 ± 0.45	50.73 ±0.40	51.88 ± 0.47	53.40 ± 0.91
MCH(pg)	17.16 ± 0.11	17.07 ±0.17	17.72 ± 0.21	17.37 ± 0.29
MCHC(g/dl)	33.10 ± 0.25	33.65 ± 0.14	34.15 ± 0.14	32.80 ± 1.03
PLT($\times10^3\mu l$)	1424.20 ± 32.55	1358.00 ± 46.19	1335.17 ± 53.69	1093.66 ± 47.09

Crocin and control were administered intraperitoneally once a day during 21 days. Data showed as mean ± SEM, Comparison with control, ANOVA test, n=6.

Table 2- Effect of crocin on hematological parameters after 21 days treatment in dose of 180 mg/kg

Test	Normal saline 10 ml/kg	crocin 180 mg/kg
WBC ($\times10^3\mu l$)	8.2 ± 0.8021	7.75 ± 0.9269
RBC ($\times10^6\mu l$)	8.46 ± 0.25	7.99 ± 0.25
HGB (g/dl)	14.183 ± 0.16	13.33 ± 0.40
HCT (%)	45.68 ± 0.77	42.43 ±1.27
MCV (fL)	54.12 ±1.02	53.12 ± 0.26
MCH (pg)	16.83 ± 0.42	16.7 ± 0.14
MCHC (g/dl)	31.05 ± 0.24	31.4 ± 0.19
PLT ($\times10^3\mu l$)	1031.5 ± 40.06	1250.83 ± 81.72*↑

Crocin and control were administered intraperitoneally once a day during 21 days. Data showed as mean ± SEM, Comparison with control, * $p<0.05$, Tukey Kramer test, n=6.

A 45 mg/kg dose of crocin increased LDH (IU/L), while crocin decreased ALP (IU/L) at doses of 15 and 45 mg/kg in biochemical tests (Table 3). The doses of 45 and 90 mg/kg decreased albumin level in a dose-dependent manner (Table 3). Crocin (180 mg/kg) also decreased the creatinin level (Table 4). However, the macroscopic appearance and weight of the kidney was not altered.

The target organs of the treated animals (heart, lung, liver, kidney and spleen) did not show a significant change in weight after 21 days. We did not observe pathological changes suggesting the organ toxicity. According to the report and despite the other organ containers being colorless, the formalin in liver container in 180 mg/kg crocin group was completely saffron yellowish. This happened in all crocin doses with less intensity, suggesting that the most crocin accumulation has happened in the liver. A decline in alveolar size in lungs of 180 mg/kg crocin group was also observed. Moreover, in hearts of this group myosin light chain atrophy and decrease in size of cell nuclei were seen. Despite of the excessive tubular loss of crocin that made the rats urine orange-yellow, there was not a gross pathologic change in kidney. The red pulp of spleen was congestive.

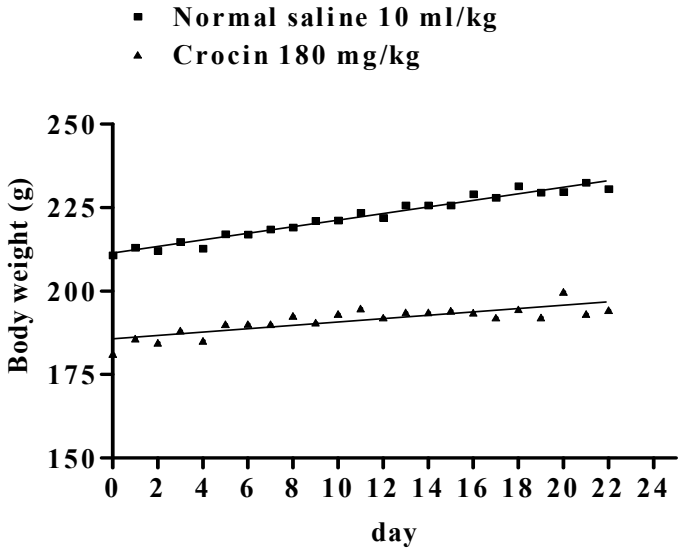

Figure 3. Effect of intraperitoneally administration of crocin on body weight. Crocin and control were administered intraperitoneally once a day during 21 days. There is a significant difference between the weight gain slope in two groups during 21 days of drug administration (*** $p<0.001$).

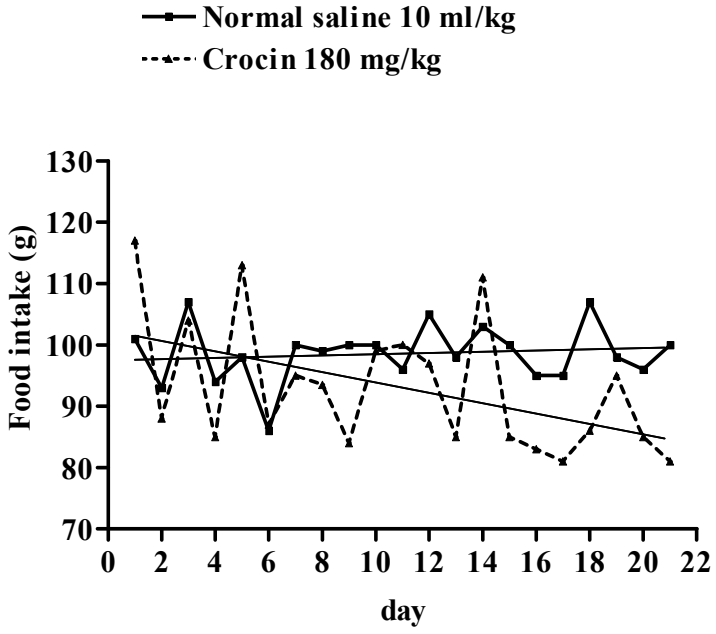

Figure 4. Effect of intraperitoneally administration of crocin on food intake. Crocin and control were administered intraperitoneally once a day during 21 days. The slope of the daily amount of food intake is relatively stable and slightly growing in normal saline group while it is downward in crocin group.

Table 3- Effect of crocin on biochemical parameters after 21 days treatment in doses from 15 to 90 mg/kg

Test	normal saline 10 ml/kg	Crocin 15 mg/kg	Crocin 45 mg/kg	Crocin 90 mg/kg
BUN (mg/dl)	24.45 ± 1.01	23.22 ± 8.88	24.08 ±1.08	22.43 ± 0.87
Creatinine (mg/dl)	0.73 ± 0.04	0.63 ± 0.02	0.67 ± 0.03	0.67 ± 0.03
Cholesterol (mg/dl)	63.33 ± 3.50	67.67 ± 2.54	63.33 ± 3.69	63.67 ± 1.04
Triglycerides (mg/dl)	66.33 ± 6.71	67.50 ± 8.35	71.50 ± 4.49	81.33 ± 11.41
SGOT (AST) (IU/L)	146.17 ± 8.54	190.83 ± 12.10	192.33 ± 29.55	147.33 ± 7.02
SGPT (ALT) (IU/L)	73.33 ± 4.02	86.83 ± 3.75	79.17 ± 4.88	80.00 ± 5.86
LDH (IU/L)	1041.78 ± 114.64	793.53 ± 110.64	1778.35 ± 312.73	905.40 ± 178.60
ALP (IU/L)	410.67 ± 20.84	315.67 ± 17.15*↓	311.33 ± 20.49*↓	384.83 ± 26.33
CPK (IU/L)	598.18 ± 124.25	629.58 ± 256.35	629.58 ± 256.35	259.39 ± 48.97
Glucose (mg/dl)	314.17 ± 4.25	378.67 ± 68.93	277.17 ± 11.47	330.17 ± 26.58
Bilirubin Total (mg/dl)	0.32 ± 0.02	0.27 ± 0.04	0.35 ± 0.08	0.30 ± 0.05
Albumin (g/dl)	3.28 ± 0.07	3.08 ± 0.06	2.85 ± 0.14*↓	2.45 ± 0.05***↓

Crocin and control were administered intraperitoneally once a day during 21 days. Data showed as mean ± SEM, Comparison with control, * $p<0.05$, *$P<0.001$, Tukey Kramer test, n=6.

Table 4- Effect of crocin on biochemical parameters after 21 days treatment at a dose of 180 mg/kg.

Test	Normal saline 10 ml/kg	Crocin 180 mg/kg
BUN (mg/dl)	28.17 ± 0.95	26.33 ± 0.88
Creatinine (mg/dl)	0.65 ± 0.03	0.48 ± 0.03**↓
Cholesterol (mg/dl)	58.67 ± 2.58	67.33 ± 1.48
Triglycerides (mg/dl)	72.00 ±16.03	65.17 ± 4.83
SGOT (AST) (IU/L)	132.83 ±11.23	179.5 ±55.42
SGPT (ALT) (IU/L)	65.33 ± 7.00	59.67 ± 5.05
LDH (IU/L)	968 ±156.07	1151.5 ± 188.75
ALP (IU/L)	512.67 ±54.00	604.33 ±37.76
CPK (IU/L)	550.00 ± 66.40	667.83 ± 104.21
Glucose (mg/dl)	369.17 ± 65.6	296.5 ± 34.12
Bilirubin Total (mg/dl)	0.67 ± 0.21	0.52 ± 0.05
Albumin (g/dl)	2.96 ± 0.08	2.99 ±0.08

Crocin and control were administered intraperitoneally once a day during 21 days. Data showed as mean ± SEM, Comparison with control, **$P<0.01$, Tukey Kramer test, n=6.

Conclusion

In summary, our study confirmed that crocin seems to be a practically low-toxic agent. However, further studies are necessary, to confirm this evidence.

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