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# Subacute Toxicity of *Crocus Sativus* L. (Saffron) Stigma Ethanolic Extract in Rats

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**Abstract:** The medicinal properties attributed to *Crocus sativus* L. (saffron) are extensive. The safety of saffron is important in relation to its medicinal applications. This study was performed to elucidate the possible toxic effects of ethanolic extract of *Crocus sativus* L. stigma on liver, kidney and some hematological parameters in rats. Wistar rats were randomly assigned into four groups of eight animals each. Group 1 was treated with ISS as control and Groups 2 to 4 were treated with extract administered daily for 2 weeks intraperitoneally in doses of 0.35, 0.70 and 1.05 g $kg^{-1}$, respectively. Body weight of the animals were recorded on the first, seven and final days of the experiment. The haematological studies include total RBC count, total WBC count, Hb, %HCT, MCV, MCH and MCHC. Biochemical/serum profile studies include ALT, AST, urea, uric acid and creatinine. Tissue specimens of the liver and kidneys were subjected to histological examination using standard hematoxyline-eosin staining. The extract caused significant reductions in the Hb and HCT levels and total RBC count, although it showed any dose-dependent effect. Total WBC count showed significant dose-dependent increases in extract treated rats. Significant dose-dependent increased values of AST, ALT, urea, uric acid and creatinine were seen. Microscopically, there were mild to severe hepatic and renal tissue injuries supporting the biochemical analysis. The results indicated that extract of *Crocus sativus* L. stigma is toxic in high doses.

**Key words:** *Crocus sativus* L., stigma, ethanolic extract, Subacute toxicity, rat

## INTRODUCTION

Saffron (dried stigmas of *Crocus sativus* L.) belongs to the Iridaceae family and is utilized by most people, either for medicinal or culinary purposes. It has been reported that *C. sativus* has hypolipaemic, anti-inflammatory, antidiabetic, antioxidant and anticancer effects. Moreover, *C. sativus*, is applicable for treatment of nervous disorders, spasms and asthma[1-3]. The clinical findings suggest that saffron is a safe and effective antidepressant[4]. Pharmacology studies have confirmed an anticonvulsant activity in the extract of saffron[1,5]. A potential deterrent to medicinal use of saffron comes about because contradictory information related to saffron toxicity has appeared. Some reports indicate that saffron is non-toxic[6-8]. By contrast, Crocetin, a carotenoid isolated from the saffron, has been found to be a teratogen[9]. It is often said that very high doses of saffron can cause abortion and possible toxic symptoms[10]. However, the safety of saffron is important in relation to its therapeutic actions. Therefore, this study was designed to characterize the possible toxic effects of ethanolic extract of *C. sativus* (Saffron) stigma on liver, kidney and some hematological parameters in normal rats.

## MATERIALS AND METHODS

**Plant:** The saffron was used in this study was dedicated by Novin Zaferan Co (Mashhad, Iran) and was identified by the Department of Cultivation and Development of Institute of Medicinal Plants, Tehran, Iran.

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**Preparation of the extract:** In the maceration method, 10 g of stigmas were macerated in 500 mL ethanol (80 v/v) for three days. The mixture was subsequently filtered and concentrated under reduced pressure at 40°C. The extract yield was 51% w/w.

**Animals:** Male Wistar rats with body weight ranging from 210-240 g, were procured from Pasteur Institute of Iran and maintained in an air conditioned room (25±1°C) with a 12 h light: 12 h dark cycle. Standard pellet diet and water were provided ad libitum. Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the United States guidelines (United States National Institutes for Health publication no. 85-23, revised in 1985).

**Biochemical determinations:** The haematological studies include total RBC count, total WBC count, Hb, %HCT, MCV, MCH, MCHC[11]. The activities of serum AST and ALT were measured by using commercially available kits (Boehringer Mannheim, Mannheim, Germany). Urea in the plasma was estimated by using the diagnostic kit based on the method of Fawcett and Scott[12]. Uric acid in the plasma was estimated by using the diagnostic kit based on the enzymic method described by Caraway[13]. Creatinine in the serum was estimated using the diagnostic kit based on the methods of Teitz[14].

**Experimental design:** Four groups of animals were used, each of which contained 8 rats. Group 1 including control animals to which isotonic saline solution (ISS, 10 mL $kg^{-1}$) was injected intraperitoneally. Groups 2-4 were extract treated rats received ethanolic saffron extract administrated intraperitoneally in doses of 0.35, 0.70 and 1.05 g $kg^{-1}$ b.w. (equivalent to 10, 20 and 30 percentages of LD50 value), respectively with once dose in a volume of 10 mL $kg^{-1}$ daily for 2 weeks. Body weight of the animals was recorded at the beginning and after 7 and 14 days of experiment. Haematological and biochemical/serum profile parameters were estimated at the end of experiment. Animals of the different groups were sacricified by cervical dislocation 6 h after the last injection and liver and kidneys were removed. Tissue specimens were fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin-eosin.

**$LD_{50}$ experiment:** Male wistar rats, weighing about 210-240 g were divided into five groups of six animals each. The test substance was administered in the doses of 1, 2, 3, 4 and 5 g $kg^{-1}$ b.w. intraperitoneally in a volume of 10 mL $kg^{-1}$ to the groups, respectively. Then the rats were observed continuously for 1 hr, intermittently for 6 hr and at the end of 48 hr for any gross behavioral changes and deaths. Food consumption, feces and urine were also examined at 2 hr and then at 6 hr intervals for 48 hr.

**Statistical analysis:** Student's -test and a probability level of $P<0.05$ were chosen as the criteria for statistical significance. Values reported are mean±standard error of the mean (SEM).

## RESULTS

**Effect on body weight of rats:** The perusal of Table 1 reveals that the extract produced significant decrease in the body weight in all the three doses (0.35, 0.70 and 1.05 g $kg^{-1}$ b.w.). This effect was dose-dependent at 14th day.

**Effect on haematological parameters:** The results of the study (Table 2) indicated marked significant decreases in the Hb and HCT levels and total RBC count in the extract treated rats, although it showed any dose- dependent effect. The extract caused significant dose-dependent increases in total WBC count. Values for the other parameters stay without significant variations.

**Effect on serum biochemical parameters:** Results of the effect, of graded doses of ethanolic extract of *C. sativus* stigmas on serum biochemical parameters of rats in intraperitoneal route are shown in Table 3. The marker enzymes ALT and AST were significantly increased by the extract. At the same time, the extract treated rats had increased levels of serum urea, uric acid and creatinine. Ethanolic saffron extract showed a dose-dependent effect on said parameters.

Table 1: Effect of the ethanolic extract of *C. sativus* L. stigma on body weight in rats

| Experimental group | Dose (g $kg^{-1}$) | Body weight (g) 0 day | 7 day | 14 day |
|---|---|---|---|---|
| ISS (Conrol) | - | 229.8±3.3 | 234.4±5.5 | 237.4±4.1 |
| Extract | 0.35 | 230.1±2.5 | 224.8±3.2* | 215.1±2.2* |
| Extract | 0.70 | 230.8±3.1 | 220.7±5.2* | 201.6±3.9** |
| Extract | 1.05 | 233.6±2.8 | 215.5±3.5* | 179.8±5.4*** |

Values are mean±SEM; (n = 8); *: p<0.05; **: p<0.01; ***: p<0.001: Compared with the control group

Table 2: Effect of the ethanolic extract of *C. sativus* L. stigma on haematological parameters in rats

| Experimental group | Dose (g $kg^{-1}$) | Total WBC count | Total RBC count | Hb (g $dL^{-1}$) | HCT (%) | MCV (fl) | MCH (pg) | MCHC (g $dL^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| ISS (Conrol) | - | 6.1±0.7 | 8.3±0.3 | 14.9±0.6 | 42.1±0.4 | 49.7±0.8 | 18.1±0.2 | 35.5±0.2 |
| Extract | 0.35 | 8.5±0.7* | 5.9±0.1*** | 10.4±0.3*** | 32.9±0.3*** | 51.4±0.7 | 17.9±0.3 | 35.1±0.3 |
| Extract | 0.70 | 10.3±0.9** | 5.7±0.2*** | 10.1±0.2*** | 30.7±0.6*** | 50.2±0.5 | 18.3±0.1 | 36.1±0.2 |
| Extract | 1.05 | 14.9±1.1*** | 5.4±0.1*** | 10.2±0.4*** | 30.3±0.7*** | 50.8±0.4 | 18.1±0.1 | 35.3±0.3 |

Values are mean±SEM; (n = 8); *: p<0.05; **: p<0.01; ***: p<0.001: Compared with the control group

Table 3: Effect of the ethanolic extract of *C. sativus* L. stigma on serum biochemical parameters in rats

| Experimental group | Dose (g $kg^{-1}$) | Alanine transaminase (IU $L^{-1}$) | Aspartate transaminase (IU $L^{-1}$) | Urea (mg $dL^{-1}$) | Uric acid (mg $dL^{-1}$) | Creatinine (mg $dL^{-1}$) |
|---|---|---|---|---|---|---|
| ISS (Conrol) | - | 24.43±0.6 | 74.52±1.3 | 19.2±0.6 | 2.1±0.1 | 0.4±0.02 |
| Extract | 0.35 | 35.28±0.9* | 88.37±2.1* | 27.8±1.2* | 2.9±0.8* | 0.7±0.03* |
| Extract | 0.70 | 50.92±1.1** | 107.20±3.2** | 38.4±2.9** | 3.6±0.9** | 1.1±0.02** |
| Extract | 1.05 | 69.91±1.4*** | 139.58±3.1*** | 50.9±4.1*** | 4.9±1.2*** | 1.9±0.02*** |

Values are mean±SEM; (n = 8); *: p<0.05; **: p<0.01; ***: p0.001: Compared with the control group

**Histopathological studies:** Histological studies of the liver and kidneys were carried out on experimental groups, which were sacrificed after 2 weeks of the experiment. Histological parameters of the liver and kidneys were normal in control rats. Histopathology of the liver in extract (0.35 g $kg^{-1}$) treated rats showed a spectrum of changes including random small foci of degeneration and necrosis, sinusoidal dilation and loss of usual concentric arrangement of hepatocytes, congestion and dilation of veins (Fig. 1A). In the histopathological studies of extract (0.70 g $kg^{-1}$) treated liver, there were more damages including broad areas of fatty change and necrosis in the central portions of the hepatic lobules (Fig. 1B), lymphocytic and granulocytic infiltrations in the portal areas (Fig. 1C). Histopathological examination of extract (1.05 g $kg^{-1}$) treated liver showed further injuries including periportal degeneration and necrosis, accumulation of Kupffer's cells with active phagocytosis in the periportal regions, bridging of inflamed portal tracts between different lobules, wide spread lytic necrosis and hemorrhage.

The changes in the kidneys of extract (0.35 g $kg^{-1}$) treated rats were enlargement of lining cells of tubules, fatty infiltration (Fig. 1D) and expansion of the glomeruli with hyaline proteinaceous drop-lets within urinary spaces. After 2 weeks of administration of ethanolic extract of C. *sativus* L. stigma, to group 3 (0.70 g $kg^{-1}$) rats, kidneys showed glomerular lesions as hyperemia, mild mesangial hypercellularity, thickening of glomerular basement membrane (Fig. 1E) and mesangial matrix along with synechiae. Hyaline casts (Fig. 1E) occluding the tubules with some tubular dilatation were also seen. In the kidneys of the extract (1.05 g $kg^{-1}$) treated animals, severe tubular necrosis and detachment of the cells from the basal membrane and large areas of hemorrhage and lymphocytic

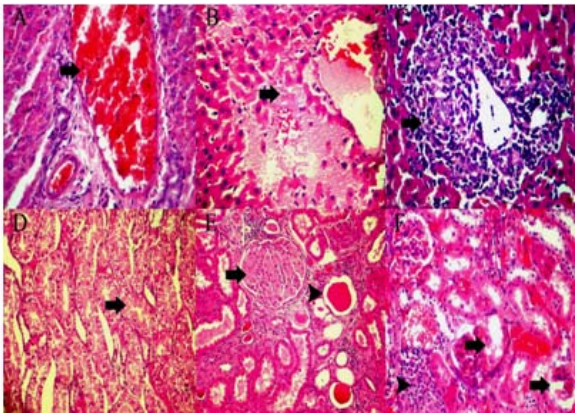


Fig. 1: Photomicrograph of the liver of extract (0.35 g $kg^{-1}$) treated rats (A): Showing congestion and dilation of portal veins (arrow) (H and E, X 320). Livers of extract (0.70 and 1.05 g $kg^{-1}$) treated rats (B and C): Showing necrosis in the central portions of the hepatic lobules and lymphocytic and granulocytic infiltrations in the portal areas (arrows) (H and E, X 320). Photomicrograph of the kidney of extract (0.35 g $kg^{-1}$) treated rats (D): Showing fatty change of lining cells of tubules (arrow) (H and E, X 100). Kidneys of extract (0.70 g $kg^{-1}$) treated rats (E): showing thickening of glomerular basement membrane (arrow) and hyaline casts in the tubules (arrowhead) (H and E, X 100). Extract (1.05 g $kg^{-1}$) treated kidneys (F): Showing severe hemorrhage and lymphocytic infiltration (arrowhead) and shrinkage and necrosis of squamous epithelial cells (arrows) (H and E, X 100)

infiltration were also prominent. Severe shrinkage (Fig. 1F) and almost complete loss of glomerular capillaries and necrosis of squamous epithelial cells (Fig. 1F), which cover the parietal layer of the Bowmen's capsule, were also conspicuous in this group.

**LD50 experiment (Behavioral effect and toxicity):** In the dose of 5 g $kg^{-1}$ b.w., mortality rate was 100%. The doses tested produced the following signs: writhing, lose of locomotion activity, ataxia and, at the highest doses administered, lethargy, hypothermia and death. The intensity of these toxic effects was dose-dependent. Four deaths were noticed with 4 g $kg^{-1}$ of *C. sativus*. Doses of 3 and 2 g $kg^{-1}$ resulted in two and one deaths, respectively. In the dose of 1 g $kg^{-1}$ b.w., there was no behavioural change up to 4 h and no mortality was observed up to end of 48 h. Intraperitoneal median lethal dose (LD50) value of ethanolic extracts was found to be 3.5 g $kg^{-1}$ b.w.

## DISCUSSION

Ethanolic extract of saffron stigma was found to significantly reduce the body weight in the rats. Decreased appetite has been shown as a clinical complication and side effect following the treatment with saffron [15,16]. Significant increase of total WBC count may be a consequence of inflammatory reactions produced in damaged tissues of extract treated animals. The results indicated that stigma ethanolic extract caused normochromic-normocytic anemia. There were no obvious hemolytic changes in the serums of the extract treated animals. We can suggest, therefore, that the anemia may be due to bone marrow suppression.

Extract treated rats have increased activities of these enzymes which reflect the hepatic damage[17]. The extract treated rats had also increased levels of serum urea, uric acid and creatinine, which are considered as significant markers of renal dysfunction[14]. Histopathological studies, showed prominent hepatic and renal tissue injuries in extract treated rats, which support the biochemical analysis. Centrilobular degeneration and necrosis of heptocytes is particularly common, as this portion of the lobule receives the least oxygenized blood and is therefore susceptible to hypoxia. Periportal degeneration and necrosis may occur following exposure to toxins[18]. Thus, the histopathological findings of the liver in our study reflect the direct and typical toxic action of the plant extract as well as severe anemia in extract treated rats.

The present results showed that ethanolic extract of *C. sativus* had toxic effects. It is reminded that saffron taken in large amounts is highly toxic[10]. However, avoiding this valuable spice might be unnecessary because the lethal dose for saffron (20.0 g) is higher than the therapeutic doses, as with a maximum daily dose of 1.5 g, so far no risks have been documented[10].

## CONCLUSION

The results indicated that extract of *Crocus sativus* L. stigma is toxic in high doses. Thus, reduction in the given doses could be preventing the toxic effects produced by this preparation. Therefore, saffron appears to have no order of toxicity when ingested in culinary and therapeutic amounts. Taken in all, the use of this plant is then supported but the precise active substance(s), site(s) and cellular and molecular mechanism(s) responsible for the said effects are still to be determined.

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