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PHYTOCHEMICAL PROFILE, ANTIOXIDANT ACTIVITY AND TOXICITY OF *CROCUS SATIVUS* L. PETALS

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Abstract

Crocus sativus L. is a medicinal plant of considerable pharmacological interest. This study investigated the phytochemical composition and assessed the acute and subacute oral toxicity of the methanolic crude extract of *C. sativus* petals in Wistar rats. Phytochemical screening revealed high levels of flavonoids, tannins, and reducing compounds, suggesting strong antioxidant potential. In acute toxicity tests, the extract was non-lethal at doses up to 5000 mg/kg, with dose-dependent hyperglycemia as the main alteration, without major short-term hepatic or renal damage. In the subacute toxicity study, low doses were well tolerated and improved lipid parameters, whereas high doses induced significant metabolic disturbances, including hyperglycemia, dyslipidemia, and increased transaminase levels, indicating hepatotoxicity. In addition to hepatotoxicity, nephrotoxicity was also observed. The data of subacute toxicity show a massive increase in urea levels, jumping from 47.63 ± 2.30 mg/dl in the control group to 126.23 ± 8.53 mg/dl in the highest dose group. This is a 165% increase with a highly significant p-value, indicating severe renal distress or failure. Hematological analysis showed overall stability, except for reduced neutrophils and erythrocyte changes at higher doses. These findings suggest that the petal extract is potentially beneficial at low doses but exhibits toxicity at higher concentrations, warranting further studies to define its therapeutic margin.

Introduction

Crocus sativus L. (family Iridaceae) is a sterile perennial herb cultivated for its dried red stigmas, saffron, a highly valued spice of culinary, medicinal, and economic importance. Likely originating from Asia Minor or the Middle East, it has spread across the Mediterranean and beyond (Bathaie and Mousavi 2010, Srivastava *et al*. 2010).

In Algeria, saffron cultivation is recent but expanding, especially in semi-arid regions. Ain Fezza (Tlemcen province) offers suitable conditions, including moderate altitude, sandy-clay soils, and rainfall supporting dryland or irrigated cultivation. Local initiatives have successfully produced saffron of international quality, highlighting its potential for regional economic development (Bemmoussat and Bouchaour 2020). Pharmacologically, the stigmas of *C. sativus* show antioxidant, anti-inflammatory, neuroprotective, antidepressant, cardioprotective, and anticancer activities, mainly linked to crocin, picrocrocin, and safranal (Rezaee and Hosseinzadeh 2013). Petals have also been reported to possess antioxidant and antidepressant activities, attributed to their flavonoid and anthocyanin content

While the stigmas of *C. sativu*s are well documented for their phytochemical and toxicological properties, the petals remain largely unexplored. In particular, no *in vivo* toxicity study has been conducted on *C. sativus* petals. Therefore, to address this knowledge gap, the present study aims to characterize the phytochemical profile of the methanolic extract of

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C. sativus petals and to evaluate its acute and subacute oral toxicity in Wistar rats.Through acute and subacute toxicity testing, we investigated its effects on biochemical parameters (hepatic, renal, and metabolic functions) and hematological indices to assess its tolerance and clarify its potential effects. This approach supports the safe use and valorization of saffron petals as a medicinal or nutraceutical resource.

Materials and Methods

Petals of *Crocus sativus* L. (saffron) were collected from the Tlemcen region, northwestern Algeria. Botanical identification was confirmed at the PPABIONUT laboratory, University of Tlemcen. A reference specimen has been retained in the PPABIONUT laboratory collection.

The study site is a saffron plot located on Mr. Chikhi Issa's farm in Aïn Fezza (34°52'38.1" N, 1°12'58.1" W; 900 m altitude), about 8 km east of Tlemcen. It is bordered by National Road N7 to the north, private farms to the south, a concrete block factory to the east, and the Ain Fezza CCLS to the west.

The petals of *C. sativus* were dried at room temperature in the absence of light and then finely ground. The powder (5 g) was macerated in 100 ml of a 70 : 30 (v/v) methanol–water mixture under orbital agitation at 100 rpm for 24 hrs. The whole mixture was first filtered through muslin cloth, followed by filtration using Whatman filter paper (Grade 1, Ø 150 mm). The filtrate was concentrated at 45°C using a rotary evaporator until a dry extract was obtained, which was then dissolved in 0.9% saline solution (NaCl).

Phytochemicals were identified according to Trease and Evans (1989) and Harborne (1973): tannins ($FeCl_3$, blue-black), flavonoids (NaOH, yellow), reducing compounds (Fehling, red precipitate), quinones (H_2SO_4, red), terpenoids (Salkowski, reddish-brown), anthraquinones (Bornträger, pink/red). Absence of alkaloids (Mayer), saponins (foam test), and coumarins (UV after NaOH) was confirmed. For the acute toxicity study, 12 female rats were randomly divided into 4 groups of 3 rats each (n=3 per group) according to OECD Guideline 423. For the subacute toxicity study, 30 female rats were randomly divided into 6 groups of 5 rats each (n=5 per group) according to OECD Guideline 407. The difference in group sizes is due to different OECD guidelines: acute toxicity testing requires fewer animals (n=3) because it is a limit test with mortality as the endpoint, whereas subacute testing requires more animals (n=5) to assess biochemical and hematological parameters.

The acute oral toxicity of the *C. sativus* petal methanolic extract was assessed following OECD Guideline 423 (OECD 2001). Twelve female Wistar rats were divided into four groups (n = 3): the control group received 0.9% saline, while the experimental groups received 1000, 3000, or 5000 mg/kg of extract by gavage. The doses were selected based on OECD Guideline 423 recommendations. Animals were monitored daily for 14 days for clinical signs or mortality, and body weight was recorded regularly

The subacute oral toxicity of the methanolic extract of *C. sativus* petals was evaluated in accordance with OECD Guideline 407 (OECD 2008). A total of 30 female Wistar rats were divided into six groups (n = 5 per group). The control group received only 0.9% saline solution. The five experimental groups were administered different doses of the petal extract at 1.56, 15.62, 62.5, 250, and 1000 mg/kg body weight. These doses were selected according to a geometric progression (1.56×4^n) as recommended by OECD Guideline 407 for subacute toxicity studies (OECD 2008). The extract was administered by oral gavage (1 ml/100 g body weight) once daily for 28 consecutive days. Daily observations included general health status, mortality, water and food consumption, and body weight progression. At the end of the experiment, the animals were sacrificed for biochemical and hematological analyses, and macroscopic examination of the liver,

kidneys, and white adipose tissue showed no gross pathological changes (color, texture, size, or lesions) in any of the treatment groups compared to the control group.

To assess the effects of *C. sativus* petal extract on health status, biochemical and hematological parameters were measured in treated rats. Biochemical markers included high-density lipoprotein (HDL) cholesterol, total cholesterol, glucose, total proteins, aspartate aminotransferase (AST) (also called TGO), alanine aminotransferase (ALT) (also called TGP), triglycerides, urea, and creatinine. These parameters were analyzed using Spinreact kits to evaluate lipid metabolism, hepatic function, glycemia, and renal status. Hematological parameters measured included: red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), hematocrit (Ht), platelets (PLT), mean platelet volume (MPV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocytes, monocytes, eosinophils, neutrophils, and basophils. All measurements were performed using an XN-1000 automated analyzer (Sysmex, Kobe, Japan) following the manufacturer's recommendations (Affy *et al*. 2018). Data were expressed as mean ± standard deviation (SD). Group comparisons for acute and subacute toxicity tests were analyzed by one-way ANOVA followed by Dunnett's post-hoc test (Minitab 18), with significance set at $p < 0.05$

Results and Discussion

The results of the phytochemical screening are presented in Table 1. Examination of the methanolic extract of *C. sativus* petals revealed a high abundance of tannins, flavonoids, and reducing compounds, a moderate presence of quinones and terpenoids, and a low proportion of anthraquinones. This profile suggests potential value for food or nutraceutical applications while highlighting the need for further analyses to confirm the identity and role of these compounds in the overall properties of the extract. To evaluate the potential impact of the extract on the physiology of animals subjected to the acute toxicity test, several biochemical parameters were analyzed. These indicators were selected to detect possible hepatic, renal, or metabolic alterations induced by treatment. The acute administration of *C. sativus* extract, at doses up to the maximum tested level of 5000 mg/kg, was generally well tolerated. Most of the evaluated biochemical parameters remained within physiological ranges, with no evidence of major systemic toxicity. Among these parameters, blood glucose emerged as the most affected marker, showing a significant and dose-dependent increase ($p < 0.001$). This hyperglycemia may reflect an acute stress response, activation of energy metabolism, or a transient disturbance of hormonal regulation induced by the extract (Everds *et al.* 2013, Armario 2015). Although Hosseinzadeh and Sadeghnia (2005) did not specifically investigate glycemia, their work on safranal demonstrated its influence on oxidative stress, suggesting a potential link between glucose regulation and oxidative stress modulation.

To the best of our knowledge, no studies have reported immediate hyperglycemia following a single dose of *C. sativus* petal extract, which suggests this effect may be transient and specific to our experimental model.Most of the literature focuses on chronic administration, often associated with improvements in glucose and lipid metabolism. For instance, Karimi-Nazari *et al*. (2019) demonstrated a significant reduction in fasting blood glucose and HbA1c after eight weeks of daily saffron supplementation (15 mg/day) in prediabetic subjects, while Samarghandian *et al.* (2013) reported decreases in glucose, lipid levels, and oxidative stress in diabetic rats treated for four weeks. Renal markers (urea, creatinine) remained stable, indicating no acute nephrotoxicity, consistent with crocin’s reported nephroprotective effects (Naghizadeh *et al*. 2008, Zhou *et al*. 2022). Liver enzymes (ALT, AST) showed no significant changes, suggesting possible enzyme induction without cytolysis, in line with crocin’s hepatoprotective activity (Yang *et al.* 2014,

Boskabady *et al*. 2016, Altinoz *et al*. 2017). Total protein levels were unchanged, confirming preserved hepatic function.

Table 1. Phytochemical screening of the methanolic extract of *Crocus sativus* petals.

Secondary metabolites	Tests/Reagents	Hydro-methanolic extract of Crocus sativus petals
Saponins	Foam test	–
Tannins	$FeCl_3$	+++
Flavonoids	$HCl + Mg^{2+}$	+++
Quinones	NaOH	++
Anthraquinones	NH_4OH	+
Coumarins	UV fluorescence	–
Reducing compounds	Fehling's reagent	+++
Terpenoids	Chloroform + H_2SO_4	++
Alkaloids	Mayer	–
	Wagner	–

(–) not detected; (+) low presence; (++) moderate presence; (+++) high presence.

The absence of alkaloids and coumarins in our extract contrasts with previous reports (Rahbardar and Hosseinzadeh 2023). This discrepancy may be due to differences in extraction methods, plant origin, growing conditions, or harvest time. All tests were repeated twice with fresh extracts and yielded consistent results.

Table 2. Biochemical parameters – Acute toxicity (Mean ± SD) and ANOVA p-value.

Parameter	Control (Tc)	1000 mg/kg	3000 mg/kg	5000 mg/kg	p-value (ANOVA)
HDL Cholesterol (mg/dl)	41.75 ± 4.53	45.12 ± 3.00	47.56 ± 13.74	45.74 ± 4.84	0.116
Total Cholesterol (mg/dl)	35.22 ± 5.64	28.24 ± 5.58	33.79 ± 13.17	31.04 ± 14.76	0.100
Glucose (mg/dl)	121.07 ± 22.73	125.04 ± 14.89	139.28 ± 1.70	160.16 ± 21.45	0.000007
Total Proteins (g/dl)	4.01 ± 0.018	3.75 ± 0.014	3.93 ± 0.013	4.28 ± 0.131	0.081
AST (TGO) (U/L)	66.58 ± 5.40	70.00 ± 4.00	71.00 ± 18.25	65.67 ± 0.65	0.106
ALT (TGP) (U/L)	37.00 ± 2.25	38.50 ± 4.56	38.83 ± 4.02	41.25 ± 0.56	0.080
Triglycerides (mg/dl)	56.90 ± 8.53	54.44 ± 18.11	53.39 ± 0.82	50.18 ± 3.78	0.096
Urea (mg/dl)	47.22 ± 17.36	47.06 ± 5.20	48.53 ± 7.66	50.00 ± 9.00	0.649
Creatinine (mg/dl)	0.80 ± 0.0004	0.76 ± 0.0001	0.75 ± 0.0025	0.78 ± 0.0007	0.265

Overall, these results indicate that *C. sativus* extract, despite a transient rise in blood glucose, shows no significant acute systemic toxicity, supporting previous findings on safranal (Hosseinzadeh *et al*. 2013).

In the subacute toxicity study, key biochemical parameters were measured to detect physiological changes. Data (mean ± SD) were analyzed by one-way ANOVA followed by Dunnett's post-hoc test ($p < 0.05$) and are shown in Table 3. The extract produced a dose-dependent response in plasma biochemistry. At low doses (1.56 and 15.62 mg/kg), it was well tolerated, with no significant changes in transaminases or total protein levels, indicating no hepatic or metabolic toxicity. Improvements in lipid profile were noted, with increased HDL-cholesterol and reduced total cholesterol, suggesting a protective effect on lipid metabolism. At high doses (250 and 1000 mg/kg), the extract induced marked hepatotoxicity, evidenced by a significant ALT increase ($p < 0.001$), decreased total protein levels, and disrupted lipid and glucose homeostasis (reduced HDL-cholesterol, elevated total cholesterol, and dose-dependent hyperglycemia). As shown in Table 3, glucose increased from 128.74 to 231.38 mg/dl ($p < 0.001$), indicating stress-

induced hyperglycemia (Everds et al. 2013). Urea increased by 165% (47.63 to 126.23 mg/dl) and creatinine rose from 0.45 to 1.50 mg/dl ($p < 0.001$), indicating severe renal dysfunction (Ferguson *et al.* 2008). Elevated ALT/AST and decreased total protein reflect hepatocellular injury and impaired synthetic function (Giannini *et al*. 2005).These results align with Hosseinzadeh and Sadeghnia (2005), suggesting saffron's safety at low doses but possible oxidative stress–related toxicity at high doses. The biphasic lipid response (HDL elevation at low doses, reduction at high doses) further supports this dual effect. Overall, acute and subacute studies indicate good tolerance at low doses; the extract was well tolerated up to 5000 mg/kg in acute administration, with hyperglycemia as the only notable effect. Hematological analysis showed no significant changes in white and red blood cells, platelets, and leukocyte subpopulations (Table 4), consistent with Karimi *et al*. (2004), who reported no subacute hematotoxicity of saffron extract in rats.

Table 3. Biochemical parameters (Mean ± SD) and ANOVA p-values (Subacute toxicity).

Parameter	Control	1.56 mg/kg	15.62 mg/kg	62.5 mg/kg	250 mg/kg	1000 mg/kg	p-value (ANOVA)
Glucose (mg/dl)	128.74 ± 2.45	128.77 ± 10.96	121.37 ± 1.61	157.46 ± 12.36	198.18 ± 4.77	231.38 ± 7.97	2.68×10^{-9}
Total Cholesterol (mg/dl)	65.99 ± 5.66	56.60 ± 2.44	43.27 ± 3.20	61.40 ± 6.47	66.96 ± 5.81	72.37 ± 0.79	7.99×10^{-5}
HDL Cholesterol (mg/dl)	50.06 ± 3.23	49.80 ± 2.13	72.74 ± 6.99	56.69 ± 4.92	25.72 ± 1.03	13.82 ± 0.61	3.72×10^{-9}
Creatinine (mg/dl)	0.45 ± 0.02	0.45 ± 0.05	0.50 ± 0.05	0.70 ± 0.04	1.13 ± 0.12	1.50 ± 0.10	1.25×10^{-9}
Urea (mg/dL)	47.63 ± 2.30	46.07 ± 3.29	50.21 ± 1.14	82.60 ± 3.72	100.33 ± 5.07	126.23 ± 8.53	1.93×10^{-10}
Triglycerides (mg/dl)	85.10 ± 3.10	83.07 ± 2.92	77.08 ± 8.01	69.79 ± 6.65	53.65 ± 4.54	45.31 ± 1.71	1.63×10^{-6}
Total Proteins (g/dl)	4.07 ± 0.19	4.89 ± 0.47	4.30 ± 0.32	4.32 ± 0.26	3.13 ± 0.16	2.67 ± 0.10	4.26×10^{-6}
AST (TGO) (U/L)	49.00 ± 1.75	47.17 ± 3.59	43.25 ± 2.75	50.33 ± 1.77	68.03 ± 2.89	86.63 ± 3.87	2.31×10^{-9}
ALT (TGP) (U/L)	36.58 ± 2.38	35.00 ± 0.50	40.25 ± 3.36	43.43 ± 3.27	79.92 ± 2.67	110.05 ± 3.81	1.68×10^{-12}

Table 4. Effects of the methanolic extract of *Crocus sativus* petals on hematological parameters in rats during the acute and subacute toxicity study.

Parameter	Control (Mean ±Var)	1.56 mg/kg	15.62 mg/kg	62.5 mg/kg	250 mg/kg	1000 mg/kg	p-value (ANOVA)
White blood cells (WBC, 10^3/µl)	10.67 ± 0.072	10.54 ± 0.516	9.75 ± 0.112	10.03 ± 0.260	10.42 ± 0.199	9.97 ± 0.044	0.1526
Platelets (10^3/µl)	722 ± 16.00	725 ± 475.00	748.5 ± 20.25	755.5 ± 492.75	730.67 ± 554.33	736.33 ± 356.33	0.2192
Neutrophils (%)	23.03 ± 2.26	23.40 ± 0.64	27.10 ± 2.71	20.67 ± 0.143	21.80 ± 4.81	24.13 ± 1.66	0.00253
Lymphocytes (%)	73.80 ± 1.12	73.03 ± 0.123	74.27 ± 1.76	74.83 ± 10.58	77.10 ± 1.83	75.90 ± 32.53	0.5592
MPV (fl)	6.03 ± 0.063	6.20 ± 0.25	7.10 ± 1.00	8.37 ± 0.863	5.95 ± 0.203	7.20 ± 0.81	0.01097
Red blood cells (RBC, 10^6/µl)	8.10 ± 0.396	8.26 ± 0.212	7.67 ± 1.206	7.63 ± 0.320	8.42 ± 0.757	8.67 ± 0.0002	0.4247
Hematocrit (HCT, %)	43.93 ± 10.20	43.85 ± 0.203	40.30 ± 2.56	42.03 ± 1.85	43.75 ± 0.903	46.30 ± 0.0001	0.01320
MCH (pg)	18.58 ± 0.058	17.95 ± 0.0025	17.95 ± 3.063	19.10 ± 0.93	19.55 ± 0.0225	20.15 ± 0.0025	0.03589
Monocytes (%)	1.85 ± 0.0043	1.76 ± 0.0057	2.00 ± 0.01	2.10 ± 0.201	2.21 ± 0.069	2.16 ± 0.255	0.4337
Basophils (%)	0.37 ± 0.0004	0.36 ± 0.0001	0.35 ± 0.0013	0.32 ± 0.0003	0.35 ± 0.0009	0.37 ± 0.0007	0.2122
MCHC (g/dl)	34.57 ± 0.143	31.70 ± 0.09	30.90 ± 0.49	31.37 ± 0.663	35.60 ± 0.01	35.40 ± 0.01	4.62×10^{-8}
Hemoglobin (Hb, g/dl)	14.37 ± 1.29	13.45 ± 0.303	12.15 ± 0.063	12.80 ± 0.28	14.60 ± 3.24	15.45 ± 0.0025	0.00887

Values are expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA. NS: not significant difference ($p > 0.05$); S: significant difference ($p < 0.05$); HS: highly significant difference ($p < 0.001$). Abbreviations: WBC = white blood cells, RBC = red blood cells, Hb = hemoglobin, HCT = hematocrit, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, MPV = mean platelet volume.

A selective decrease in neutrophils was observed at certain doses, suggesting a potential effect on specific leukocyte subpopulations, although this phenomenon requires further investigation. A limitation of this study is the absence of a reference toxicant (positive control) to validate the sensitivity of the biochemical and hematological assays. However, all assays were performed

using validated methods (Spinreact kits for biochemistry, Sysmex XN-1000 for hematology) following the manufacturers' protocols, and control group values were consistent with expected physiological ranges.

Regarding total protein levels, the control value (4.07 ± 0.19 g/dl) was below the reference range for healthy Wistar rats (5.5-7.6 g/dl) (Giknis and Clifford 2008). Nevertheless, the significant dose-dependent decrease to 2.67 ± 0.10 g/dl at 1000 mg/kg ($p < 0.001$) confirms severe hypoproteinemia, indicating impaired hepatic synthetic function.

Low doses (1.56 and 15.62 mg/kg) of *C. sativ*us petal extract were well tolerated. However, high doses (250 and 1000 mg/kg) induced significant toxicity, including severe nephrotoxicity (165% increase in urea, from 47.63 ± 2.30 to 126.23 ± 8.53 mg/dL, $p < 0.001$) and hepatotoxicity (elevated ALT, AST, and decreased total protein). Therefore, the extract is safe only at low doses, whereas high doses are clearly toxic and nephrotoxic. Phytochemical analysis revealed high levels of tannins and flavonoids, and absence of alkaloids, saponins, and coumarins (Table 1).

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