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Effect of safran, safranal and crocin which are active ingredients of Saffron (Crocus) on erythrocyte fragility and hematological parameters in carbon tetrachloride intoxicated rats

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ABSTRACT

Carbon tetrachloride is a toxic chemical and well known for its carcinogenic property. Intoxication with carbon tetrachloride causes serious liver injury and it is used as an experimental model for triggering liver related diseases. Saffron (*Crocus*) species are well known bulbous ornamental and aromatic plants. Many of the saffron species are used in ethnomedicinal practices. In addition both saffron species and their ingredients are also subject to scientific research. In this study 72 rats were divided into 9 groups (n=8 in each group). Groups were set as control, olive oil, carbon tetrachloride, safran, safranal, crocin, carbon tetrachloride + safranal, carbon tetrachloride + crocin. Administrations were conducted for 7 days. Erythrocyte osmotic fragility and some selected hematological parameters were assessed. Results state significant increase in erythrocyte fragility due to carbon tetrachloride and amelioration due to active ingredients of saffron. Among hematocrit, hemoglobin and red blood cell count parameters only significant alteration was observed in safran with an increase in red blood cell count. Different administration schemes can be evaluated in future studies in order to assess detailed impact of saffron constituents on hematological parameters and erythrocyte integrity.

Key Words: Saffron, carbon tetrachloride, erythrocyte fragility, safran, safranal, crocin

Introduction

Carbon tetrachloride (CCL_4) is a transparent, non-flammable toxic chemical which may quickly evaporate from its liquid form (1). Experimental or unintended exposure to CCL_4 causes serious hazards to organisms. It is a cytotoxic xenobiotic. Cellular damage due to CCL_4 is caused by augmented lipid peroxidation. CCL_4 transforms into CCL_3 which is a free radical (2). Main outcome of CCL_4 exposure is hepatotoxicity. Experimental studies reveal that CCL_4 causes hepatic fatty degeneration, mononuclear cell infiltration, fibrosis, degeneration in hepatocytes, cirrhosis and cancer (3). Since liver degeneration due to CCL_4 resembles cirrhosis advance in humans, CCL_4 is widely used as a chemical agent in experimental studies in rodents. Oxidative stress is an important counterpart in this liver toxicity caused by CCL_4. Various synthetic and natural compounds mainly from plants were tested against CCL_4 toxicity (4).

Crocus sativus is a member of *Iridaceae* plant family. It is known as saffron. It is widespread in Turkey and Iran and used as food and for cosmetic purposes. In addition, it is used in ethnomedicine as anti-spasmodic, aphrodisiac, anti-convulsant, stomachic and in treatment of diseases such as dysentery, cholera, depression and insomnia. Scientific studies on this plant and its active ingredients revealed that it has anti-depressant, anti-convulsant, anti-inflammatory, anti-diabetic and anti-tumoral activity (5-8). Saffron and its ingredients; safranal, crocin and crocetin are proven to exert anti-oxidant activity in many studies (9-14).

In order to test activity hazardous or protective activity of different chemicals and products of natural origin there are various existing models such as cytotoxic, anti-tumoral and anti-oxidant activity. Erythrocyte osmotic fragility is another test for assessment of membrane integrity and resistance against damage triggered by osmosis (15). Although it is simple and easy to assess, it

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provides valuable information. Processes occurred such as increased lipid peroxidation or augmented protein carbonylation due to exposed chemicals or disease situations attenuate durability of erythrocytes against such hazardous effects. Studies concerning impact of oxidative stress on osmotic fragility of erythrocytes are present in literature. Suboh et al (16) studied protective effect of some plants used in traditional medicinal practices on erythrocyte fragility triggered by oxidative stress due to H_2O_2 administration on erythrocytes *in vitro* and observed a protective activity. In scientific literature *in vivo* studies also reveal an attenuation of strength and integrity of erythrocytes due to increased oxidative stress as well as due to reduced anti-oxidant capacity. Kolanjiappana et al found that (17) erythrocytes of cervical cancer patients became more prone to damage due to insufficient anti-oxidant status in osmotic fragility test. Therefore, evaluation of osmotic strength of erythrocytes provides a valuable model in testing possible harmful conditions *in vitro* as well as *in vivo*.

In this study it was aimed to test effect of active ingredients of *Crocus* extracts on erythrocyte fragility and hematological parameters in carbon tetrachloride intoxicated rats.

Materials and methods

Animals: In this study Wistar albino male rats were placed in standard cages. Room temperature and humidity were kept constant within standard limits. 12h dark/12 light period was administered. Water and standard pellet food were given *ad libitum*. Ethical permission was obtained from Yuzuncu Yil University Animal Experiments Local Committee. All experimental procedures were conducted according to Helsinki Declaration.

Study design: 72 rats were divided into 9 groups (n=8 each). In each group following administrations were applied.

Group 1: Control group (standard pellet food + water + i.p. saline injection for 7 days),
Group 2: Olive oil group (1 ml/kg i.p. at 7th day),
Group 3: CCL_4 (1 ml/kg i.p., dissolved in olive oil in 1:1 ratio at 7th day),
Group 4: Safran (100 mg/kg/day orally for 7 days),
Group 5: Safranal (100 mg/kg/day i.p. for 7 days,
Group 6: Crocin (100 mg/kg/day i.p. for 7 days),
Group 7: CCL_4 (1 mL/kg, i.p., dissolved in olive oil in 1:1 ratio at 7th day) and Safran (100 mg/kg/day i.p. for 7 days),
Group 8: CCL_4 (1 mL/kg i.p., dissolved in olive oil in 1:1 ratio at 7th day) and Safranal (100 mg/kg/day, i.p. for 7 days),
Group 9: CCL_4 (1 mL/kg i.p., dissolved in olive oil in 1:1 ratio at 7th day) and Crocin (100 mg/kg/day, i.p. for 7 days).

24 hours following CC_{44} administration (8th day) rats were sacrificed. Blood samples were obtained from venous blood under anesthesia.

Hematological measurements: Venous blood was withdrawn into EDTA containing tubes. Number of red blood cells (RBC), hematocrit (HCT) and hemoglobin (HGB) were measured with an automated device suitable for animal studies - Abacus Junior Vet 5, Australia).

Erythrocyte fragility: Osmotic fragility of erythrocytes was assessed with a spectrophotometer at 546 nm (Prime-Ev, BPC). Blood samples were incubated for a day in ambient temperature. 30 μL from erythrocyte package of those incubated blood samples were added onto solutions containing buffers (Na_2HPO_4 and NaH_2PO_4) in order to achieve suitable pH conditions and different concentrations of NaCl (0.4 and 0.5 % NaCl). After 30 minutes of incubation at room temperature, blood containing solutions were centrifuged at 3000 rpm for 5 minutes. Absorbance of supernatant fractions was evaluated with spectrophotometer (18).

Statistical analysis: Results are presented as mean ± standard deviation. Kruskal-Wallis and Tukey's tests were performed for statistical analysis.

Results

Erythrocyte fragility: Erythrocyte fragility values expressed as percentage of hemolysis due to salinity for 0.4 and 0.5 % NaCl concentrations were found significantly higher for CCL_4 compared to control ($p<0.05$) (Figure 1).

Hematological parameters: Selected hematological parameters namely RBC, HCT and HGB were assessed. Results state a significant augmentation in red blood cell count in safran administered group ($p<0.05$). Other findings for RBC count state an attenuation in crocin group without statistical significance. HCT values were found highest in safran and CCL_4Crocin administered groups. Similarly with HCT values,

no significant difference was found for HGB in administration groups compared to control. Results of hematological parameters are shown in table 1.

Discussion

In this study which aimed to test effect of saffron active ingredients on erythrocyte fragility and some selected hematological parameters in CCL_4 intoxicated rats, a significant increase in erythrocyte osmotic fragility in CCL_4 administered group and an amelioration with administered ingredients was found. In addition, a significant increase in RBC value was observed for safran administered group.

Erythrocytes are used extensively in oxidative stress research since they contain hemoglobin and polyunsaturated fatty acids in their membranes (19). CCL_4 is a toxic xenobiotic and known to increase both oxidative stress and erythrocyte osmotic fragility (20). In experimental procedures a single dose administration of CCl_4 (1 mL/kg, i.p.) is known to exert erythrocyte damage i

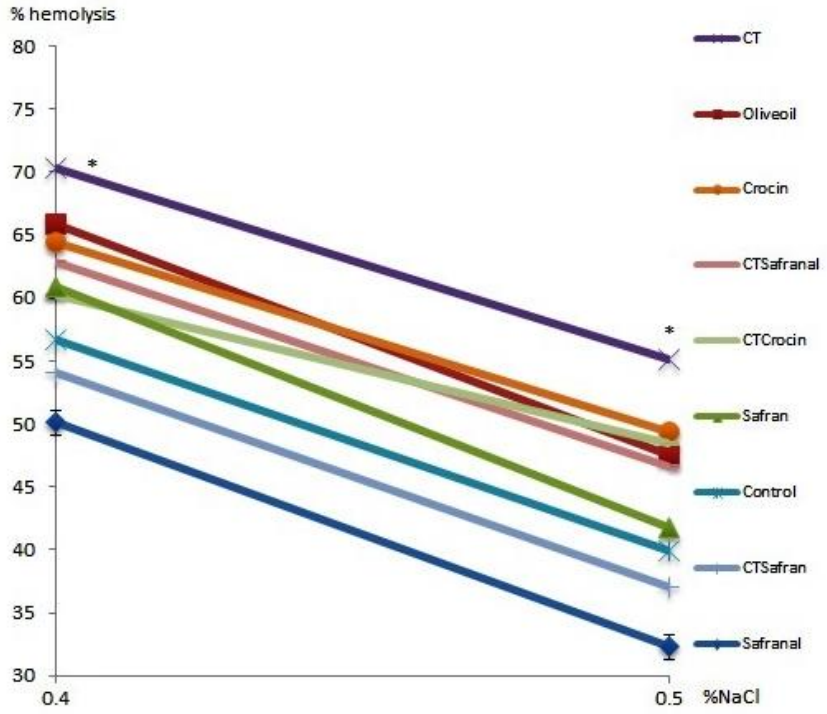

Fig. 1. Effect of CCL4, safran, safranal, crocin and concomitant use of them with CCL4 on erythrocyte osmotic fragility. Values are presented in percent hemolysis. *=statistically significant compared to control ($p<0.05$). CT= Carbon tetrachloride.

Table 1. Results of different administrations on some selected hematological parameters

	RBC(x106/mm3)	HCT (%)	HGB (g/dL)
Control	7.21±0.47	46.30±2.37	14.25±0.92
Olive oil	7.97±0.23	47.50±1.42	15.55±0.35
CCL_4	8.18±0.44	50.55±3.46	16.50±0.71
Safran	8.71±0.62*	53.45±4.88	16.50±0.70
Safranal	6.49±0.25	51.65±1.07	13.15±0.49
Crocin	7.62±0.21	46.40±1.71	15.00±0.14
CCL_4Safran	8.19±0.14	50.65±1.64	15.95±0.35
CCL_4Safranal	7.68±0.42	48.90±4.38	14.90±0.99
CCL_4Crocin	8.11±0.24	53.65±2.90	16.05±0.17

RBC: Red blood cell, HGB: hemoglobin, HCT: hematocrit. Values are presented as mean±standard deviation. (n=8 for each group). CCL_4: carbon tetrachloride. Significance is accepted as $p<0.05$. *=compared to control.

Wistar albino rats (19). Erythrocyte fragility is an indicator of cellular damage to organism due to exposed chemicals or disease situations. Increased erythrocyte osmotic fragility was observed in experimental hypothyroidism in Sprague Dawley rats (21) and in human sleep apnea patients (22). Some of those disease situations are also known to be related with augmented oxidative stress in patients. Preventing oxidative stress caused by molecules such as CCL_4 and protection of erythrocytes against fragility is intensively studied. Research focus on amelioration of this parameter via administered substances which are mostly of natural origin such as plant extracts or active ingredients (23). Anti-hemolytic activity of saffron and its ingredients are also studied. Khalili et al (15) found that extraction of aerial parts of *Crocus caspius* strongly inhibited H_2O_2-induced hemolysis in mice blood. This finding is similar with our results indicating an amelioration of hemolytic effect of CCL_4 on erythrocytes.

In this study RBC, HGB and HCT were selected since they are known to be affected by some ingredients of saffron. Such selected hematological parameters in our study revealed no significant effect of CCL_4 and other ingredients except safran with a significant increase in RBC count. This finding is contradictory with results of Saba et al (24). They have found microcytic hypochromic anemia due to CCL_4 administration. However their administered dosage of CCL_4 was higher (1.25mL/kg) compared to ours (1 mL/kg). An insignificant attenuation of HGB and HCT were observed due to crocin in a study by El-Beshbishy et al (25). In our study, no such effect was observed in crocin and an insignificant decline in RBC count and HGB value in safranal group was observed. Difference between these two studies may because of administered doses. Dosage of crocin in the mentioned study was 2 times higher (200 mg/kg) than our dosage (100 mg/kg).

Saffron and its active ingredients are known to exert protective effect against oxidative stress (26) and genotoxicity (27). Therefore it is expected to observe protective effect against damage on somatic cells as well as erythrocytes. In our study saffron active ingredients namely safran, safranal and crocin exerted protective effect on CCL4 induced augmentation in erythrocyte fragility. Longer exposure of such ingredients may also yield significant results on hematological parameters.

Result of this present study reveals a protective effect of saffron active ingredients namely safran, safranal and crocin on CCL_4 induced erythrocyte fragility. No significant alteration in selected hematological parameters (RBC, HCT and HGB) due to CCL4 and listed active ingredients except safran may be due to short administration period. Research studies covering more time periods may be useful for assessment of protective effect of saffron ingredients on this model.

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