ORIGINAL ARTICLE

Eastern Journal of Medicine

East J Med 27(3): 424-431, 2022
DOI: 10.5505/ejm.2022.34356

Saffron and Saffron Ingredients Like Safranal and Crocin's Cytoprotective Effects on Carbon Tetrachloride Induced Liver Damage

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ABSTRACT

This study aims to investigate the liver-protective effects of saffron and saffron components like crocin and safranal against acute liver injury induced by carbon tetrachloride (CCI_4) in rats.

72 male Wistar Albino rats were used in the study. The rats were divided into nine groups: 8 in each group. The rats were sacrificed at the end of the seventh day with high excretion (exsanguination). The protective effect of saffron, safranal, and crocin against CCI_4-induced acute liver injury was evaluated histopathologically.

In the study, balloon degeneration, apoptotic cells, and sub-massive necrosis were seen in hepatocytes as a result of- the effect of CCI_4. These histopathological changes were hardly seen in groups in which CCI_4 was administered with crocin and saffron. On the other hand, in the group where safranal and CCI_4 were given together, changes similar to the histopathological changes seen in the CCI_4 group were observed.

It was concluded that crocin and saffron have a hepatoprotective effect in the experimental acute liver injury caused by CCI_4, but the safranal has no protective effect on the liver.

Keywords: Saffron, Crocin, Safranal, Carbon tetrachloride

Introduction

The liver is an organ that regulates a wide variety of biochemical processes such as detoxification of toxic chemicals and drugs (1). A significant part of liver damage occurs due to oxidative stress that causes the formation of free radicals. One of the substances causing damage to the liver is carbon tetrachloride (CCI_4), which causes the production of free radicals. The CCI_4 molecule is converted into the carbon trichloride (CCl3) free radical by cytochrome-P450. By adding oxygen molecules to this radical, a carbon trichloride peroxyl (CCI_3OO) radical is formed, which initiates lipid peroxidation. Toxic lipid peroxidation products resulting from the peroxidation of fatty acids cause membrane damage and cause cell death (2). However, experimental studies indicate that CCI_4 causes fatty liver, balloon degeneration, necrosis, fibrosis, cirrhosis, and cancer (3). CCI_4 is widely used as a chemical agent in experimental studies in rodents. Oxidative stress is the main factor in CCI_4-induced liver toxicity and many natural and synthetic substances derived from plants have been tested against CCI_4toxicity (4-8).

Crocus sativus L., also known as saffron, is a cultivated perennial plant from the Iridaceae family. These plants are grown in Iran, Turkey, and Spain and are used for cosmetics, food, and medical purposes (9, 10). Some studies have shown that saffron and its components have anti-carcinogenic, anti-inflammatory, and anti-depressant properties (10-16). In addition, crocin, safranal, and crocetin, which are the main constituents of saffron, and saffron have been proven to have antioxidant properties in many studies (17-22). Iranshahi et al. (23) histopathologically showed the liver protective effect of saffron against CCI_4 toxicity and Omidi et al. (24) histopathologically showed the liver protective effect of saffron against acetaminophen toxicity. On the other hand, Ziaee et al. (25) showed that safranal, a saffron component, has a toxic effect on the liver. Although the

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Received: 02.07.2021, **Accepted:** 01.02.2022

hepatoprotective effects of saffron and crocin against CCI4-induced hepatotoxicity have been previously reported in the literature, the hepatoprotective effects of safranal still need to be investigated. In addition, the hepatoprotective effects of these substances were not compared histopathologically.

In this study, we aimed to investigate histopathologically the hepatoprotective effects of saffron and its active constituents, crocin, and safranal, in a CCI4-induced acute liver injury model in rats.

Materials and Methods

Chemicals: Olive oil from Sigma-Aldrich USA (O1514), CCl4 from Sigma-Aldrich USA (289116), saffron from Sigma-Aldrich USA (S8381), safranal from Sigma-Aldrich USA (W338907), and crocin from Sigma-Aldrich USA (17304) were obtained.

Test Animals: The study was carried out in Yuzuncu Yil University Experimental Medicine and Research Center by the decision of the Local Ethics Committee of the Animal Experiments of Yuzuncu Yil University dated 25.12.2015 and numbered 2015/560.

In our study, 72 male Wistar Albino rats 16-20 weeks old weighing between 180 and 250 g were used. The rats were placed in plastic cages. The room temperature and humidity were kept constant within the standard limits. The 12-hour dark / 12-hour light period was applied. The rats were given standard rat feed and tap water.

Study Groups: The rats were divided into 9 groups: 8 in each group (n=72). Two extreme samples were formed. The first extreme was Group 1, saline and oil was administered, the second extreme was Group 3, and $CCI_4$was administered. Olive oil was given together with CCI_4 because it is an emulsifying agent and it is not toxic to the liver. The histopathological changes in the other groups were compared with these groups. The substances applied to the groups, their application forms, doses, and durations are summarized in Table 1.

Group 1 (n = 8): Serum physiological (SF) group received standard pellet feed, water, and oral SF for 7 days.

Group 2 (n = 8): Olive oil (1 ml/kg) was administered intraperitoneally (IP) as a single dose on the 7th day.

Group 3 (n = 8): CCl4 (1 ml/kg 1:1 ratio in IP and olive oil) was administered as a single dose on the 7th day.

Group 4 (n = 8): Saffron (100 mg/kg/day) was administered enterally with the orogastric gavage method once daily for 7 days.

Group 5 (n = 8): Safranal (100 mg/kg/day, IP) was administered once daily for 7 days.

Group 6 (n = 8): Crocin (100 mg/kg/day, Rope) was administered once daily for 7 days.

Group 7 (n = 8): CCI_4 (1 ml/kg, 1:1 ratio in IP and olive oil) as a single dose on the 7th day and saffron (100 mg/kg/day) with orogastric gavage method enteral once a day for 7 days were administered.

Group 8 (n=8): CCI_4 (1 ml/kg 1:1 ratio in IP and olive oil) as a single dose on day 7 and Safranal (100 mg/kg/day, IP) once daily for 7 days were administered.

Group 9 (n = 8): CCI_4 (1 ml/kg 1:1 ratio in IP and olive oil) as a single dose on day 7 and Crocin (100 mg/ kg/day, IP) once daily for 7 days were administered.

Histopathological Evaluation: At the end of the seventh day, rats were sacrificed by exsanguination and their livers were removed. Small incisions were made for better detection of the liver. The liver tissues were fixed in 10% formalin solution. One day later, the tissues were embedded in paraffin blocks after being monitored on an automatic vacuum tissue tracking device by Leica ASP® 300 (Leica microsystems, Germany). Leica RM® 2135 (Leica microsystems, Germany) branded rotary microtome device and 4 μm thick sections were taken from prepared paraffin blocks. These sections were stained with Hematoxylin-Eosin (H&E) for normal histopathological evaluation, Masson-Trichrome (MT) for evaluating liver fibrosis, and reticulin for evaluating the reticulin network forming the liver roof.

The preparations were evaluated histopathologically in Olympus BX53F (Olympus, Tokyo, Japan) light microscope in the laboratory. Then, they were photographed with the Olympus E-330 camera. At the histopathological evaluation, ten high power fields (x400) were randomly selected, and mean balloon degeneration, apoptotic hepatocyte, and mitosis were counted in a large zoom area. In addition, the liver was examined for fibrosis, necrosis, and anointment.

Statistical Analysis: In our study, descriptive statistics; median, mean, standard deviation, and minimum and maximum values were used. Continuous measurements were examined with the Shapiro-Wilk (n<50) test and nonparametric tests were applied because the measurements were

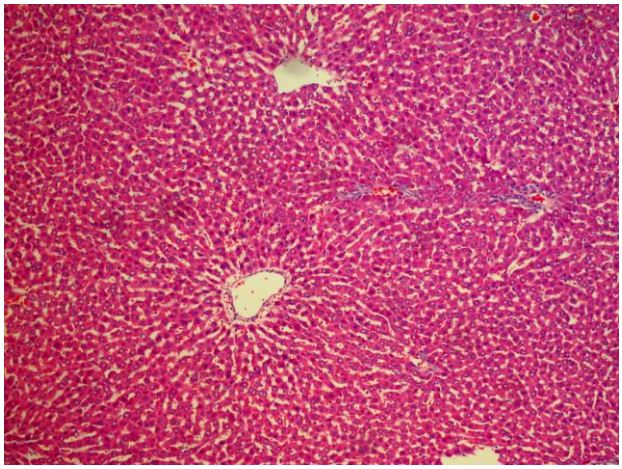

Fig. 1. Group 1 (Saline) (H&E x10)

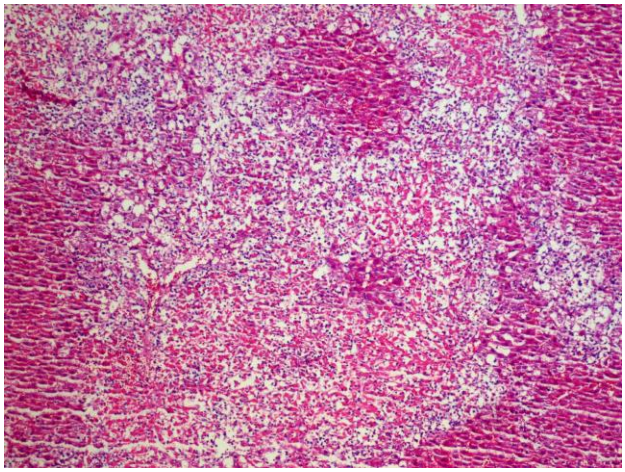

Fig. 2. Group 3 (CCl_4) (H&E x10)

not normally distributed. "Kruskal Wallis Test" was used since the data we obtained in our evaluation were not normally distributed. "Bonferroni post-hoc test" test was used to determine the difference in groups. The statistical significance level was taken as 1% and SPSS (IBM SPSS for Windows, ver.20) was used for the calculations.

Results

Monitoring The Study Groups: The rats did not die during the study in any study group.

Histopathological Evaluation: In Group 1 and Group 2, normal histological features of liver tissue were observed. Balloon degeneration, apoptotic hepatocyte, and mitosis were rarely observed (Figure 1). In Group 3, histopathologically, sub-massive necrosis and acute inflammation of neutrophilic leukocytes mixed with necrotic cells were observed in these necrosis areas. Intensive balloon degeneration was observed in the hepatocytes, which was more intense in the centrilobular area (Figure 2).

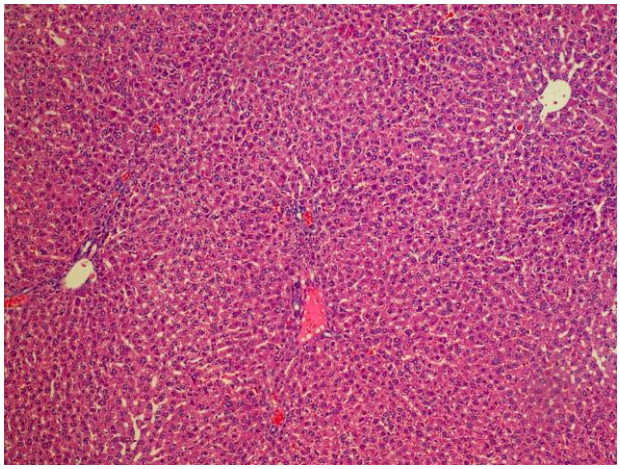

Fig. 3. Group 4 (Saffron) (H&E x10)

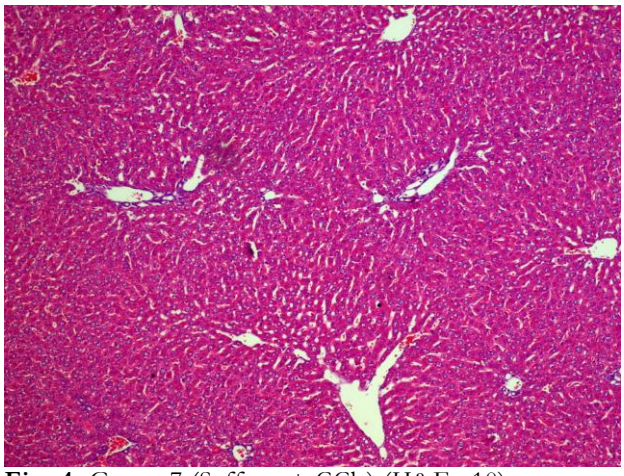

Fig. 4. Group 7 (Saffron + CCl_4) (H&E x10)

In Group 3, there was a significant increase in the number of balloon degeneration, mitosis number, and apoptotic hepatocyte count compared to Group 1 and Group 2 ($p<0.01$).

In Group 4, Group 5, and Group 6, there was no statistically significant difference in the apoptotic hepatocyte, balloon degeneration, and mitosis compared to Group 1 and Group 2 ($p>0.01$) and no histopathological changes were observed in liver tissue (Figure 3).

There was no statistically significant difference in the number of apoptotic cells, the number of mitosis, and the number of balloon degeneration in Group 7 compared to Group 1, Group 2, and Group 4 ($p>0.01$). When compared with Group 3, balloon degeneration, mitosis number, and apoptotic hepatocyte count were significantly lower ($p < 0.01$) in Group 7. In addition, necrosis foci seen in Group 3 were not observed in Group 7 (Figure 4).

While there was a statistically significant increase in apoptotic cell count and balloon degeneration in Group 8 compared to Group 1, Group 2, and

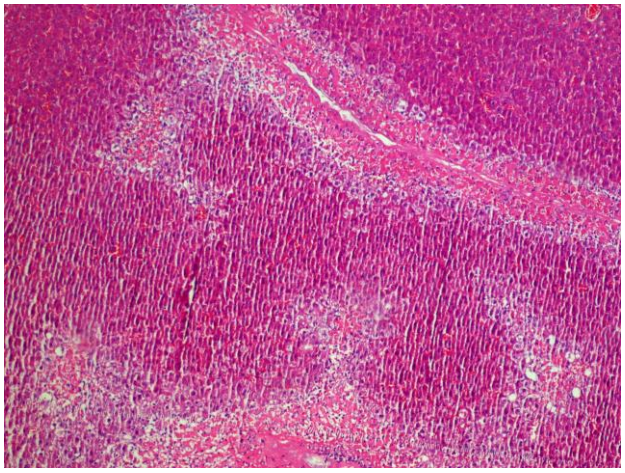

Fig. 5. Group 8 (Safranal + CCl_4) (H&E x10)

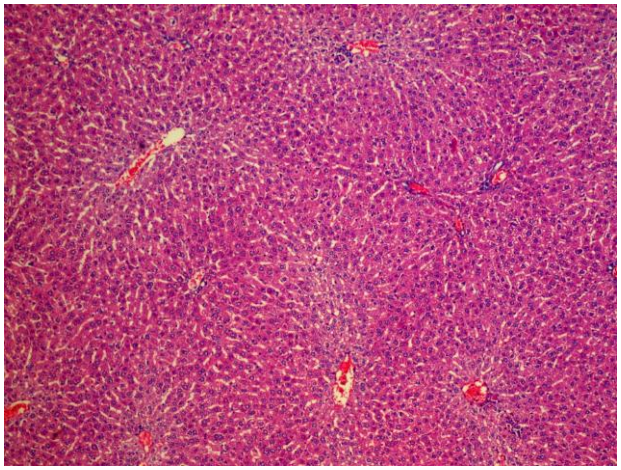

Fig. 6. Group 9 (Crocin + CCl_4) (H&E x10)

Group 5 ($p<0.01$), no statistically significant difference was detected in the number of mitosis ($p>0.01$). In comparison with Group 3, there was no statistically significant difference in balloon degeneration and apoptotic hepatocyte count ($p> 0.01$), but mitosis count was statistically significantly different ($p<0.01$). In addition, sub-massive necrosis and neutrophil leukocytes accompanying this necrosis were observed in group 8, similar to the histopathological changes seen in group 3 (Figure 5).

There was no statistically significant difference in the number of apoptotic cells, mitosis, and balloon degeneration in Group 9 compared to Group 1, Group 2, and Group 6 ($p > 0.01$). Compared to Group 3, balloon degeneration, mitosis and apoptotic hepatocyte count were statistically significantly lower ($p < 0.01$) in Group 9. Notably, necrosis foci seen in Group 3 were not seen in Group 9 (Figure 6).

In the search done with MT staining for evaluating fibrosis in the liver, no fibrosis was observed.

In the staining with reticulin to evaluate the roof of the liver, it was observed that the roof was damaged in the CCl_4 group.

The mean balloon degeneration, mean apoptotic hepatocyte count and mean mitosis in groups are summarized in Table 2.

Discussion

In this study, we investigated the hepatoprotective effects of saffron and safranal and crocin, which are saffron components, in the experimentally induced acute liver injury with CCl_4 in rats and compared the results.

Grizzi et al. detected hepatocyte necrosis in the centrilobular zone 2 hours after CCI_4 administration to the rats. In this study, hydropic degeneration and vacuolization were observed in the hepatocytes located near the centrilobular zone. After a day, these findings were reported to be more severe (26). In our study, the liver of the rats in the CCI_4 group was examined after 24 hours and evaluated histopathologically. In the liver in the CCI_4 group, common balloon degeneration and sub-massive necrosis were observed, especially in the centrilobular region, consistent with previous studies.

Saffron is a plant that has very low toxicity and has proven beneficial in many diseases. Natural antioxidants are not only ideal for removing free radicals, but also for the belief that natural products are healthier and safer than synthetic ones. Both in vivo and in vitro antioxidant properties of saffron extracts and saffron components such as crocin, crocetin and safranal have been shown (27).

Iranshahi (23) et al. found that the rate of necrosis occurring in liver damage in different doses with CCl4 in rats decreased in the groups in which saffron extract was given, revealing the hepatoprotective effect. Omidi et al. (24) found that in liver toxicity with acetaminophen in rats, saffron significantly reduced severe necrosis and inflammation in the liver. Hosseinzadeh et al. (28) showed the analgesic and anti-inflammatory effects of saffron leaf and pellet in an experiment on rats. Shati et al. (29) showed that the destructive effects of aluminium on rat liver were decreased by saffron at a molecular and biochemical level. In our study, in parallel with these studies, it was observed that saffron had a protective effect against the hepatotoxic effect of CCI_4.

Table 1. Application Methods, Doses, and Duration of The Substances Administered To Groups

	Administered substance	Application method	Dose	Duration
Group 1	SP	IP	1 ml/kg	For 7 days
Group 2	Olive oil	IP	1 ml/kg	On 7th day
Group 3	CCI_4	IP	1 ml/kg	On 7th day
Group 4	Saffron	Orogastric lavage	100 mg/kg/day	For 7 days
Group 5	Safranal	IP	100 mg/kg/day	For 7 days
Group 6	Crocin	IP	100 mg/kg/day	For 7 days
Group 7	Saffron	Orogastric lavage IP	100 mg/kg/day	For 7 days
	CCI_4		1 ml/kg	On 7th day
Group 8	Safranal	IP	100 mg/kg/day	For 7 days
	CCI_4	IP	1 ml/kg	On 7th day
Group 9	Crocin	IP	100 mg/kg/day	For 7 days
	CCI_4	IP	1 ml/kg	On 7th day

Abbreviations: CCl_4: Carbon tetrachloride, SP: Serum physiological, IP: Intraperitoneal

Table 2. Descriptive Statistics and Comparison Results

	Group	Median	Mean	SD	Min.	Max.	*p.
Mean number of apoptotic hepatocyte at an HPF	Saline	.05 C	.05	.05	.00	.10	.001
	Olive oil	.10 C	.14	.05	.10	.20	
	CCl_4	7.50 B	8.00	2.39	4.00	12.00	
	Saffron	.10 C	.09	.08	.00	.20	
	Safranal	.10 C	.11	.04	.10	.20	
	Crocin	.05 C	.05	.05	.00	.10	
	Saffron + CCl_4	.10 C	.10	.08	.00	.20	
	Safranal + CCl_4	9.50 A	10.00	2.73	7.00	14.00	
	Crocin + CCl_4	.10 C	.09	.06	.00	.20	
Mean number of mitosis at an HPF	Saline	.10 B	.09	.06	.00	.20	.006
	Olive oil	.05 B	.05	.05	.00	.10	
	CCl_4	.20 A	.23	.13	.10	.40	
	Saffron	.00 B	.04	.05	.00	.10	
	Safranal	.00 B	.04	.05	.00	.10	
	Crocin	.05 B	.05	.05	.00	.10	
	Saffron + CCl_4	.00 B	.02	.05	.00	.10	
	Safranal + CCl_4	.05 B	.05	.05	.00	.10	
	Crocin + CCl_4	.00 B	.05	.08	.00	.20	
Mean balloon degeneration the number at an HPF	Saline	.00 B	.01	.04	.00	.10	.001
	Olive oil	.00 B	.01	.04	.00	.10	
	CCl_4	73.00 A	74.00	10.49	60.00	91.00	
	Saffron	.00 B	.03	.05	.00	.10	
	Safranal	.00 B	.03	.05	.00	.10	
	Crocin	.00 B	.02	.05	.00	.10	
	Saffron + CCl_4	.30 B	.29	.11	.10	.40	
	Safranal + CCl_4	71.00 A	70.13	9.20	55.00	82.00	
	Crocin + CCl_4	.10 B	.10	.08	.00	.20	

* Significance levels according to Kruskal-Wallis Test results. SD: Standart Deviation

A, B, C: It shows the difference between groups according to the Bonferroni Post Hoc pairwise comparison test

Bandegi et al. (30) showed that saffron and crocin are protective against oxidative damage of chronic stress in the brain, liver, and kidney. Jhaneshwari et al. (31) found that crocin significantly corrected hepatic and antioxidant enzymes against hepatotoxicity caused by cyclophosphamide in rat liver. In a study on liver toxicity of beryllium chloride, crocin was found to decrease the level of enzymes showing liver toxicity (32). By these studies, in our study, hepatoprotective effect with crocin was observed.

Boroushaki et al. (33) showed that safranal had a protective effect on hexachlorobutadiene-induced nephrotoxicity in rats. On the other hand, Ziaee et al. showed that safranal produced toxicity in the liver and that saffron reduced this toxic effect (25). In our study, although it did not damage the liver alone, it did not show any hepatoprotective effect against the hepatotoxic effect of CCI_4.

In our study, it was observed that CCI_4 -induced liver damage was significantly prevented by saffron and crocin. Despite these effects of saffron and crocin, it was observed that safranal had no hepatoprotective effect. This study was performed to investigate which component(s) of saffron is known to have a hepatoprotective effect that causes that. In addition to confirming the hepatoprotective effect of saffron in other studies, we concluded that crocin is the main component responsible for this effect since this effect is only observed in saffron constituents. Thanks to these properties, there is a need for clinical studies showing the effects of crocin or saffron on hepatotoxicity before giving many drugs that may cause liver damage. However, since the cost of crocin is low compared to saffron and does not cause any side effects in the liver, we think that crocin can be used before giving toxic drugs to the liver for hepatoprotective effect. Although the hepatoprotective effect seen with saffron and crocin was not observed with safranal, further studies were needed to investigate the effects of different doses. In addition, we think that other active components that contribute to the hepatoprotective effect of saffron and the synergistic effect of crocin and safranal should also be investigated.

As a result, in the groups where SP, olive oil, saffron, safranal, and crocin were given alone, no histopathological changes were found in the livers of the rats. In addition, CCI_4 was found to cause an increase in balloon degeneration and apoptotic hepatocyte count reflecting liver damage, and also to sub-massive necrosis in some lobules. Furthermore, when safranal was given with CCI_4, it was found that there was no hepatoprotective effect compared to the CCI_4 group, but it did not show any toxicity when given alone. Crocin and saffron were found to have a hepatoprotective effect because they significantly eliminated many histopathological changes including necrosis reflecting liver damage.

Acknowledgment: This research is funded by Yuzuncu Yil University Scientific research Projects under the serial number TTU-2016-5031.

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