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Biochemical and Inflammatory Parameters in the Protective Effect of Crocin on Hepatorenal Toxicity Induced by Nicotine and Ethanol in Adolescent Rats

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Abstract

Introduction: Nicotine and ethanol are both associated with significant oxidative stress, inflammation, and tissue damage in the liver and kidney. Heavy use of tobacco and alcohol leads to serious health consequences such as cardiovascular and cerebrovascular diseases, peptic ulcers, and various cancers. Crocin, a carotenoid compound derived from saffron (Crocus sativus), has attracted attention due to its potent antioxidant and anti-inflammatory properties. Given its potential, this study focused on evaluating the protective effects of crocin on liver and kidney dysfunction induced by nicotine and ethanolsubstances known to have toxic effects on these vital organs. It seems that crocin, with its biological activities, can play a role in reducing these adverse effects.

Materials and Method: 60 male Wistar rats (aged 21 days) were randomly divided into six groups: control, nicotine and ethanol, nicotine and ethanol (10 mg/kg crocin), nicotine and ethanol (20 mg/kg crocin), nicotine and ethanol (30 mg/kg crocin), and a group receiving 30 mg/kg crocin. Crocin was administered daily by gavage for three weeks. After the last day of the experiment, rats in all groups were anesthetized with CO2. Then, liver tissue was removed and serum was prepared. After this treatment period, important biochemical indices of kidney and liver function such as serum creatinine, blood urea nitrogen (BUN), alkaline phosphatase (ALk-P), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and the inflammatory factor TNFα were measured in adolescent mice.

Results: The results showed that the ethanol and nicotine treated group showed a significant increase in malondialdehyde (MDA) and nitrite in the liver and kidney compared to the control group. Also, the ethanol and nicotine treated groups showed a significant decrease in total thiol and SOD and catalase enzyme activities in the liver and kidney compared to the control group. The results also showed that all liver enzymes including alkaline phosphatase (ALk-P), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and the inflammatory factor TNFα increased significantly in nicotine and ethanol treatment compared to the control group. The results of this study showed that crocin significantly improves liver and kidney function against nicotine and ethanol-induced damage by reducing inflammatory factors, oxidative stress, and liver enzyme activity, and by increasing the activity of SOD and catalase enzymes and thiol groups.

Conclusion: Crocin shows a strong protective effect against nicotine and ethanol-induced liver and kidney damage in adolescent rats by reducing oxidative stress, inflammation, and tissue structure reconstruction.
Keywords: Crocin, Nicotine, Ethanol, Liver, Kidney

Introduction

The harmful consequences of alcohol and tobacco usage are two of the major threats to global health (1). Nicotine, primarily through tobacco and electronic nicotine delivery devices, is frequently used alongside alcohol or other abused drugs (2). Adolescence is a common time for tobacco and alcohol use, with nearly 90% of adult smokers starting before the age of 18 (3, 4). Heavy tobacco and alcohol use has been related to major health problems such as cardiovascular and cerebrovascular illnesses, gastric ulcers, and different malignancies, particularly in the neck, head, liver, and esophagus (1, 5). Alcohol and nicotine abuse is linked to rewarding, genetic, and possibly analgesic effects of tobacco products. The high rate of co-morbidity can be related to pharmacodynamic and pharmacokinetic interactions, as well as the potential enhancement of analgesic and rewarding effects (1, 6, 7).

Nicotine is absorbed into body tissues and metabolized by the liver, converting 70-80% to cotinine through oxidation. Additionally, the kidney excretes it via tubular secretion and glomerular filtration, contributing to 5% of the total clearance. Nicotine is eliminated via tubular secretion and glomerular filtration, with the possibility of reabsorption based on the pH level of the urine. On the other hand, cotinine has less renal clearance and is extensively reabsorb able via tubules (8, 9). Although the liver uses three different pathways to metabolize alcohol: microsomal ethanol oxidation system (MEOS), alcohol dehydrogenase (ADH), and catalase enzymes. These processes produce toxic products like reactive oxygen species (ROS) and acetaldehyde making organs like the liver more susceptible to alcohol-induced damage through unclear mechanisms (10, 11). Therefore, the liver and kidney, these two vital organs in the body, are exposed to the toxicity caused by the consumption of nicotine and alcohol.

Smoking is a major risk factor for chronic kidney disease (CKD). Nicotine exacerbates kidney damage by increasing atherosclerosis, angiogenesis, and glomerular damage (12). Also, the α7 nicotinic acetylcholine receptor (α7-nAChR) mediates nicotine's damaging effects on the kidneys. The α7-nAChR receptor stimulates mesangial cell proliferation and hypertrophy (12). Chronic nicotine exposure exacerbates acute kidney damage and raises oxidative stress, hastening the transition of acute renal injury to chronic kidney injury (13). Nicotine metabolism causes oxidative stress, cell dysfunction, and liver inflammation. It releases pro-inflammatory cytokines and activates NF-κB, worsening disorders like nonalcoholic fatty liver disease. Nicotine also alters lipid metabolism, leading to steatosis and fatty acid buildup (14-17).

Ethanol-induced liver damage often progresses via phases. The first stage is steatosis, which occurs in more than 90% of heavy drinkers. Alcoholic hepatitis is an inflammatory illness that results in liver cell loss and possible wounds. Chronic inflammation and oxidative stress cause excessive deposition of extracellular matrix proteins, which is referred to as fibrosis. Cirrhosis is the ultimate stage, which is marked by significant liver lesions and the possibility of liver failure (18-21). Alcoholic kidney injury is associated with a complex combination of ethanol-induced oxidative stress and proinflammatory alterations, which may be caused by the interaction of the kidneys with other organs such as the gut, liver, cardiovascular system, and skeletal muscles (22). Alcohol usage can contribute to kidney illness through a variety of processes, including alcohol itself or excessive

alcohol-related chemicals produced as cells break down or metabolize alcohol (22). Natural products have a long history of use in treating liver and kidney diseases due to their non-toxic nature and potential hepatorenal protective properties, often attributed to their antioxidant properties and ability to aid the endogenous antioxidant defense system (23, 24).

Crocin, a group of polyandicarboxylic acid, mono and diglucosyl esters of crostin, is the primary color found in saffron and gardenia compounds (25). Crocin has numerous medicinal effects, including anti-inflammatory, cancer cell inhibiting, and oxidative damage protection (26-28). It also has antihypertensive, antiplatelet, nephron protective, antidepressant, and antiatherosclerotic effects (29). Crocin has therapeutic potential for neurological disorders, increasing dopamine in the brain during Parkinson's disease (30). Its strong antioxidant properties neutralize free radicals and oxidative stress in the liver (31, 32). Crocin's protective properties make it a promising candidate for protecting liver and kidney function from nicotine and ethanol toxicity. Chronic use of these substances can cause severe health problems, including liver and kidney damage. Crocin seems to be a potential alternative to drugs and offers a safer and more acceptable treatment. Therefore, in our study, we investigated the effect of crocin on hepatorenal toxicity induced by nicotine and ethanol.

Materials and method

Animals

Sixty adolescent male Wistar rats (aged 21 days) were obtained from the animal house of Torbat Heydarieh University of Medical Sciences. All rats were kept in special plexiglass racks with dimensions of 30×40×15 cm and in an environment with controlled conditions in repeated periods of 12 hours of light and 12 hours of darkness (the light cycle starts at 7 am), with an approximate temperature of 23 ± 2 degrees Celsius. All experiments were performed according to the ethical guidelines set by the Ethics Committee of Torbat Heydarieh University of Medical Sciences.

Experimental design

The rats were randomly assigned to six groups (n=10): Control, nicotine and ethanol, nicotine and ethanol + 10 mg/kg Crocin, nicotine and ethanol + 20mg/kg Crocin, nicotine and ethanol + 30 mg/kg Crocin, the group receiving 30 mg/kg of Crocin. The Control group received normal saline (N/S, 2 ml/day) via gavage for 21 days. The Nicotine and Ethanol group received nicotine (2 mg/kg) for 21 days. The ethanol-receiving groups were given escalating concentrations of ethanol (10, 5, and 15%) for seven days in order to adapt the animals to ethanol, and then they were given drinking water with 20% ethanol for three weeks. The Crocin Treatment Group received crocin (30 mg/kg) via intraperitoneal (IP) injection for 21 days without nicotine or ethanol. All rats were anesthetized using CO_2 at the end of experimental period. Blood samples were collected and were placed in tubes for biochemical analysis. After blood coagulation had occurred, the samples were centrifuged at 3000 g for 15 minutes, and the collected serum was kept at -20 °C until biochemical analysis (33). At the end of the experiment, kidney and liver tissue samples were collected, washed with N/S, snap-frozen in liquid nitrogen, and kept at -80°C (34).

Biochemical Analysis

Measuring the total amount of thiol groups (SH)

DTNB reagent (Elem reagent) was used to measure thiol groups. This reagent reacts with SH groups to create a yellow complex (nitromercaptobenzoate anion) which has an absorption peak at 412 nm wavelength and its millimolar absorption coefficient is 13.6 mM-1cm-1. 50 µl of homogenous sample was added to 1 ml of buffer (Tris-EDTA 30mM), and its absorbance was read at 412 nm wavelength against Tris-EDTA buffer alone. Then, 20 µl of 10 mM DTNB reagent (in methanol) was added to it and after about 15 minutes at the laboratory temperature, the absorbance of the sample was read again (A2, the absorbance of DTNB solution was also read as a blank alone (B). The total amount of thiol groups (mM) was calculated using the following formula: (A2 -A1 -B) ×13.6 / (0.05/1.07).

Assay of compounds reactive with thiobarbituric acid (TBARS)

0.5 ml of the tissue homogenous mixture was transferred to a 10 ml centrifuge tube and then 3 ml of 1% phosphoric acid and one ml of 0.6% thiobarbituric acid solution (TBA) were added to it. The resulting mixture was heated in a boiling water bath for 45 minutes. After cooling, 4 ml of butanol was added to the above mixture, and after vortexing for one minute, the color phase of butanol was separated with the help of a centrifuge at a speed of 20,000 rpm for 20 minutes and its absorbance was read at 532 nm wavelength. The compound 1,1,3,3-tetramethoxypropane (TMP) was used as malondialdehyde (MDA) standard. In an acidic medium with a stoichiometric ratio of 1:1, MDA is released and reacts with TBA to produce a colored complex that has an absorption peak at a wavelength of about 532 nm.

Superoxide dismutase (SOD) enzyme activity assay

SOD enzyme activity is measured by the Worthington method (38). In this method, 0.1 M EDTA in 0.3 M sodium cyanide and 1.5 M NBT were added to a suitable volume of homogenous tissue in a cuvette and after mixing, it was placed at 37 degrees for 5 minutes. Then 0.12 mM riboflavin was added in 0.067 M potassium phosphate buffer with pH = 7-8 and it was placed at room temperature for 10 minutes. Absorbance was read in 5 minutes at 560 nm wavelength and specific activity was calculated in units/mg of protein.

Catalase (CAT) enzyme activity assay

Catalase enzyme activity is measured by Aebi method. In this method, absolute ethanol (0.01 ml/ml) was added to a certain volume of tissue extract and incubated in ice for half an hour. Then 10-100% Triton was added to it with a final concentration of 1%. This solution was used to measure enzyme activity. The reaction starts by adding 30 mM H_2O_2 to an appropriate volume of tissue sample extract in 50 mM sodium phosphate buffer, pH=7. Then absorbance was read in 3 minutes at 240 nm wavelength. The specific activity was calculated in units per milligram of protein.

Liver enzymes assay

The blood samples were centrifuged at 3000 rpm, 10 minutes, to separate the serum samples and stored at −20°C until analysis. The serum levels of liver enzymes ALT, AST, and ALP were measured using commercial kits (Pars Azmoon, Iran) according to the manufacturer's instructions by a serum autoanalyzer (BT-1500-A-A, Rome, Italy).

Measurement of BUN, creatinine, TNFα

For the detection of creatinine and BUN, Pars Azmoon Company diagnostic kits were used. For the detection of TNFα levels, My Bio Source kit was used.

Statistical analysis

The data obtained from biochemical evaluations were considered as mean ± standard error of the mean (SEM). GraphPad Prism version 6 was used for data evaluation. If the conditions for using parametric tests were established, the analysis of variance method was used for inter-group comparison. If $p < 0.05$, the difference in means was considered significant.

Results

1. Improvement of oxidant/antioxidant imbalance in liver tissue by Crocin

1.1 Evaluation of MDA level

Graphs A and B in Figure 1 show the results of data analysis on the measurement of MDA and nitrite in the liver in the studied groups. The mean values of MDA and nitrite are shown in Table 1. The group that received ethanol and nicotine (Nic-Eth) showed a significant increase in MDA as an index of lipid peroxidation and nitrite compared to the control group (P<0.001 for all). While the groups treated with two doses of 20 and 30 mg/kg crocin showed a significant decrease in MDA and nitrite (P<0.01 and P<0.001, respectively).

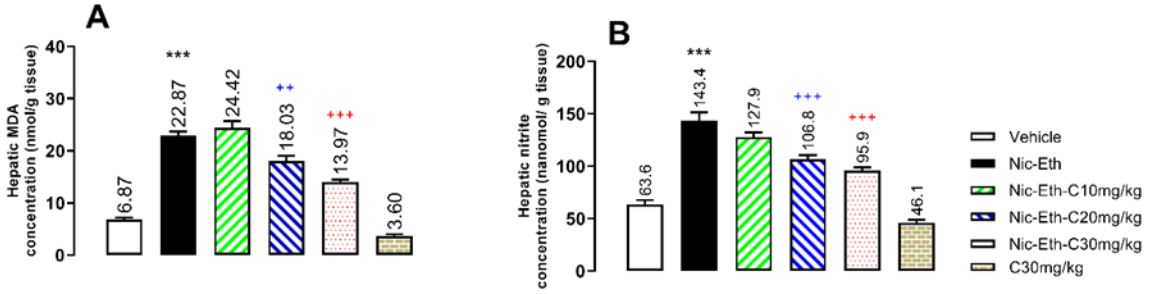


Figure 1. Assessment of oxidative profile in liver tissue. p<0.05, p>0.01** and ***p>0.001 compared to the control group and p<0.05^{+}, p>0.01^{++} and $^{+++}$p>0.001 compared to the Nic-Eth group. Data is shown as average. Statistical comparison between groups was done by ANOVA test and Tukey's post hoc test (the number of animals in each group was 10).*

Table 1. Effects of ethanol, nicotine, and crocin (4 weeks) on biochemical marker levels, Data showed as mean±SEM.

Groups	Vehicle	Nic-Eth	Nic-Eth-C10mg/kg	Nic-Eth-C20mg/kg	Nic-Eth-C30mg/kg	C30mg/kg
MDA liver	6.865±0.3510	22.87±0.8003	24.42±1.255	18.03±1.021	13.97±0.5404	3.600±0.4269
NO liver	63.64±4.130	143.4±7.947	127.9±4.158	106.8±3.863	95.91±2.995	46.13±2.700
Thiol liver	4.466±0.2763	1.966±0.05998	2.321±0.09133	2.485±0.1012	3.110±0.1665	4.400±0.3399
SOD liver	53.65±1.360	14.75±0.4218	19.60±1.469	27.20±1.680	37.53±0.5370	73.44±1.519
Cat liver	2.703±0.07052	1.606±0.05911	1.772±0.06758	2.030±0.06686	2.292±0.09276	2.727±0.09117
MDA kidney	8.432±0.6134	29.62±1.841	29.99±2.243	30.38±1.249	13.96±0.3130	4.889±0.3514
NO Kidney	53.70±4.964	142.0±6.012	127.9±2.708	98.50±3.490	78.17±3.449	29.11±4.602
Thiol kidney	12.14±0.5926	2.712±0.4501	4.372±0.6932	5.130±0.3095	8.113±0.2713	14.13±0.7181
SOD kidney	10.46±0.1782	2.162±0.1522	2.341±0.09018	3.360±0.3561	6.375±0.2903	12.33±0.3727
CAT kidney	1.091±0.03150	0.5598±0.04084	0.6206±0.02449	0.7039±0.01820	1.473±0.1107	2.011±0.05879
AST	103.9±7.701	245.4±12.20	181.3±9.734	147.3±11.63	129.1±15.15	87.88±5.992
ALT	3.875±0.2266	15.38±0.9246	13.13±0.3981	6.875±0.2266	5.750±0.3134	3.125±0.2266
ALk-p	400.0±32.82	857.8±40.70	583.9±56.60	527.3±34.60	406.4±40.66	236.6±6.256
BUN	0.4125±0.02950	0.7000±0.02673	0.4750±0.03660	0.4000±0.02673	0.4375±0.02631	0.2500±0.04226
Cr	36.75±0.3660	71.38±3.635	66.13±4.426	43.25±1.567	36.75±1.820	17.25±0.3660
TNFα	6188±140.9	13250±928.4	11457±474.9	9824±397.8	7118±534.1	2170±214.1

Nic, nicotine; Eth, ethanol; C, crocin; MDA, malondialdehyde; NO, nitrite oxide; SOD, super oxide dismutase, cat, catalase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALk, alkaline phosphatase; BUN, blood urea nitrogen; Cr, creatinine; TNFα, tumor necrose factor α.

2. Determination of total SH and activity of SOD and catalase enzymes

Graphs A-C in Figure 2 show the analysis of data related to the determination of the total thiol groups and the activities of SOD and catalase enzymes in the liver. The average values are shown in Table 1. According to graphs A-C, the Eth Nic- group showed a significant decrease in the total thiol groups and the activities of SOD and catalase enzymes compared to the control group ($P<0.001$ for all). While the groups treated with two doses of 20 and 30 crocin caused a significant increase in the activities of SOD and catalase enzymes and the dose of 30 caused an increase in the amount of total thiol groups compared to the Nic-Eth group ($P<0.01$ and $P<0.001$). Treatment with crocin alone increased the amount of SOD enzyme activity and total thiol groups compared to the control group ($P<0.001$).

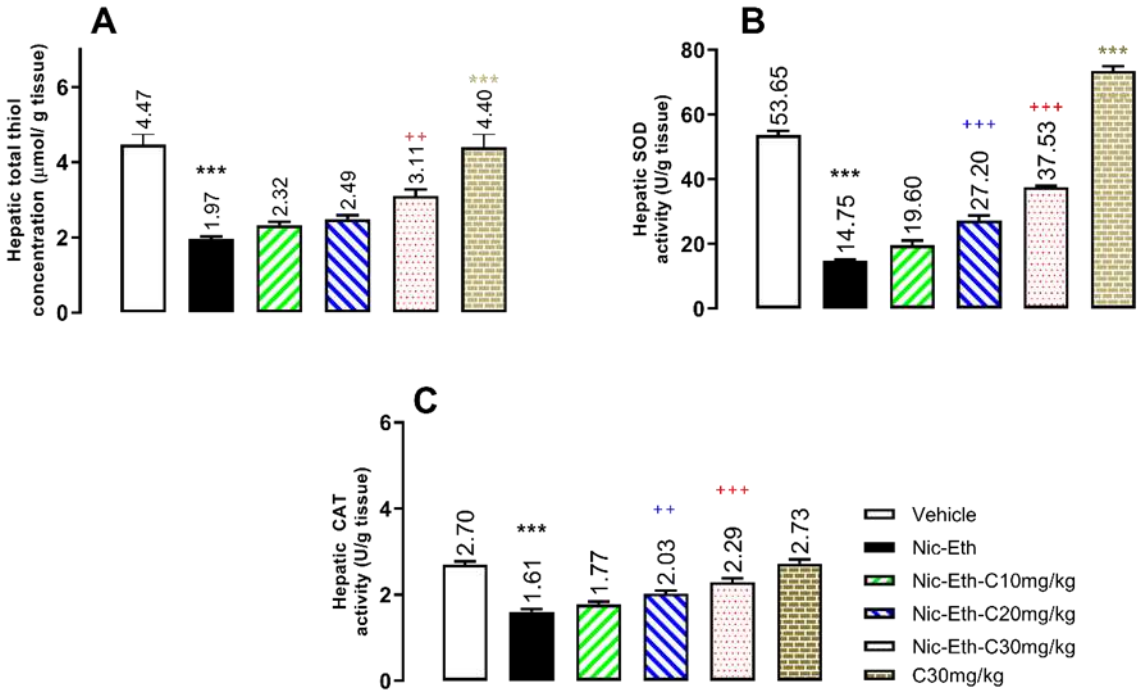


Figure 2. Assessment of oxidative profile in liver tissue. $p<0.05$, $p>0.01$** and ***$p>0.001$ compared to the control group and $p<0.05^{+}$, $p>0.01^{++}$ and $p>0.001^{+++}$ Compared to the Nic-Eth group. Data is shown as average. Statistical comparison between groups was done by ANOVA test and Tukey's post hoc test (the number of animals in each group was 10).*

3. Improvement of oxidant/antioxidant imbalance in liver tissue by Crocin

3.1 Evaluation of MDA level

Graphs A and B in Figure 3 show the results of data analysis on the measurement of kidney MDA and nitrite in the studied groups. The mean values of MDA and nitrite are shown in Table 1. According to graphs A and B in Figure 3, the Eth-Nic group showed a significant increase in MDA as an index of lipid peroxidation and nitrite compared to the control group ($P<0.001$ for all). While the groups treated with two doses of 20 and 30 crocin showed a significant decrease in nitrite compared to the Eth-Nic group ($P<0.01$ and $P<0.001$). In the case of MDA, only the dose of 30 mg of crocin caused a significant decrease compared to the Eth-Nic group ($P<0.001$). Crocin at a dose of 30 alone caused a decrease in nitrite compared to the control group ($P<0.01$).

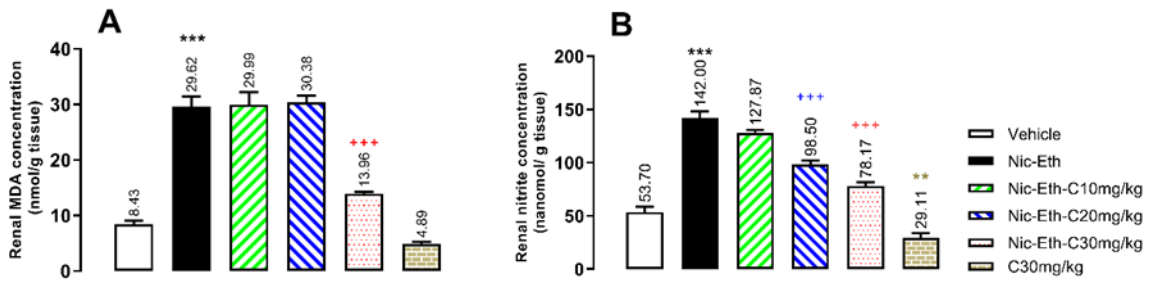


Figure 3. Assessment of oxidative profile in kidney tissue. p<0.05, p>0.01** and ***p>0.001 compared to the control group and p<0.05^{+}, p>0.01^{++} and p>0.001^{+++} compared to the Nic-Eth group. Data is shown as average. Statistical comparison between groups was done by ANOVA test and Tukey's post hoc test (the number of animals in each group was 10).*

4. Determination of total SH and activity of SOD and catalase enzymes

Graphs A-C in Figure 4 show the analysis of data related to the determination of the total thiol groups and the activities of SOD and catalase enzymes in the kidney. The average values are shown in Table 1. According to graphs A-C in Figure 4, the Eth-Nic group showed a significant decrease in the total thiol groups and the activities of SOD and catalase enzymes compared to the control group (P<0.001 for all). While the groups treated with two doses of 20 and 30 crocin caused a significant increase in the activities of SOD enzymes and the total thiol groups and the dose of 30 caused an increase in the activities of catalase enzyme compared to the Nic-Eth group (P<0.05 and P<0.001). Treatment with crocin alone caused an increase in the activities of SOD enzyme and total thiol groups compared to the control group (P<0.001).

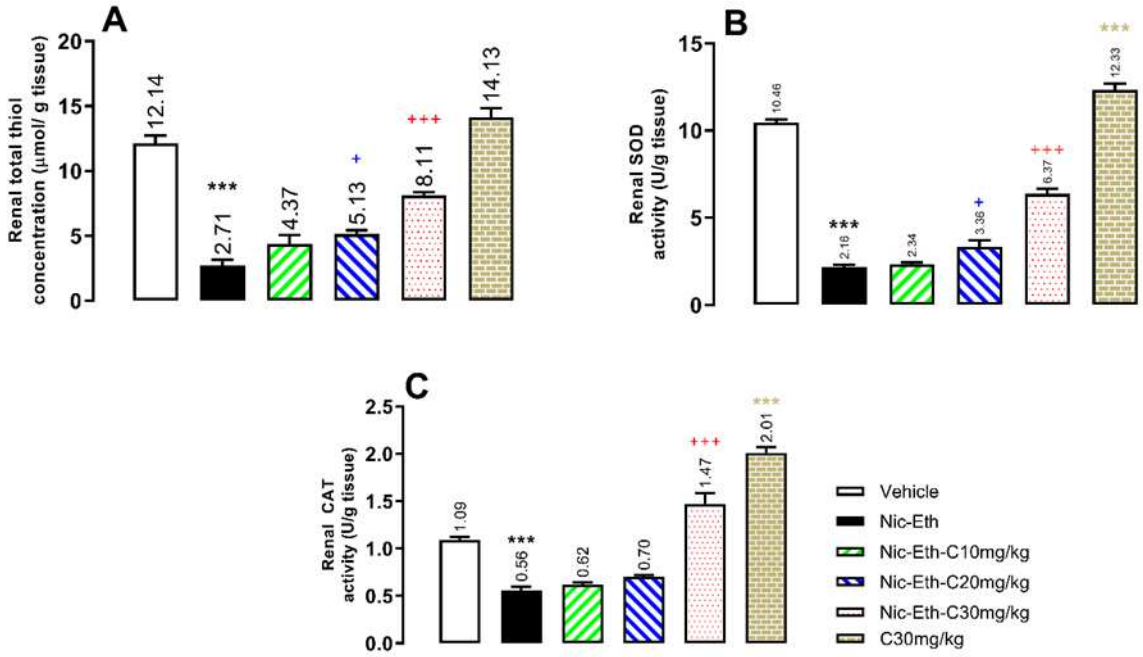


Figure 4. Evaluation of oxidative profile in kidney tissue. p<0.05, p>0.01** and ***p>0.001 compared to the control group and p<0.05^{+}, p>0.01^{++} and p>0.001^{+++} compared to the Nic-Eth group. Data is shown as average. Statistical comparison between groups was done by ANOVA test and Tukey's post hoc test (the number of animals in each group was 10).*

5. Liver enzymes

Graphs A-C in Figure 5 show the analysis of data obtained from the measurement of liver enzymes including ALk-P, ALT, and AST. The average values are shown in Table 1. According to graphs A-C, all liver enzymes including ALk-P, ALT, and AST were significantly increased in the Nic-Eth treatment group compared to the control group (P<0.001 for all values). While the treatment of the Nic-Eth groups with doses of 20 and 30 mg/kg crocin significantly decreased all liver enzymes (P<0.05, P<0.01, and P<0.001). Administration of crocin at a dose of 30 mg/kg alone reduced the ALK-P level compared to the control group (P<0.05).

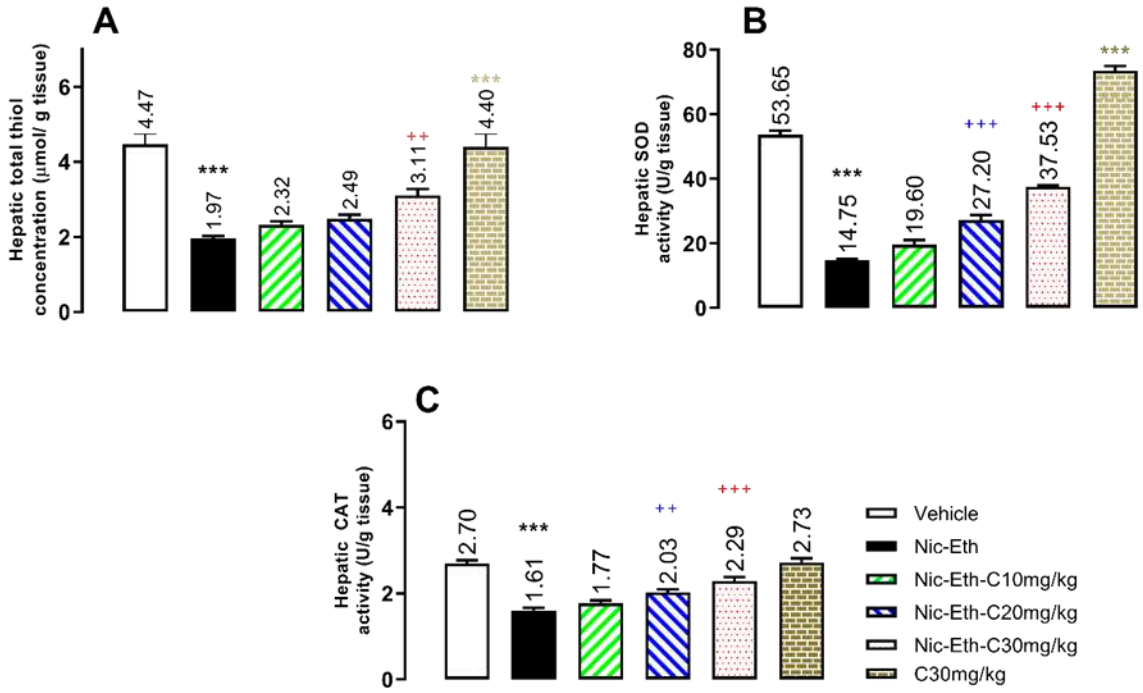


Figure 5. Evaluation of liver enzymes. p<0.05, p>0.01** and ***p>0.001 compared to the control group and p<0.05^{+}, p>0.01^{++} and p>0.001^{+++} compared to the Nic-Eth group. Data is shown as average. Statistical comparison between groups was done by ANOVA test and Tukey's post hoc test (the number of animals in each group was 10).*

6. Determination of renal function

Graphs A and B in Figure 6 show the analysis of data related to the measurement of BUN and creatinine in the liver in the studied groups. The average values are shown in Table 1. According to Graphs A and B, the BUN and creatinine levels in the Nic-Eth group were significantly higher than in the control group (P<0.001). Treatment with all three doses of crocin reduced BUN and two doses of 20 and 30 reduced creatinine levels compared to the Nic-Eth group (P<0.001). Receiving a high dose of crocin reduced BUN compared to the control group (P<0.001).

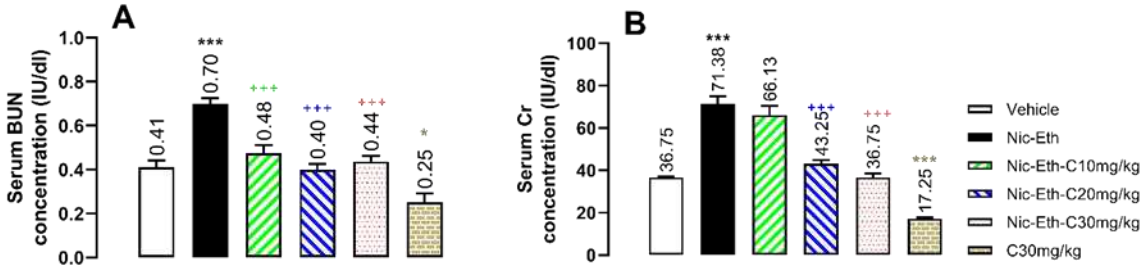


Figure 6. Evaluation of renal failure. p<0.05, p>0.01** and ***p>0.001 compared to the control group and p<0.05^{+}, p>0.01^{++} and p>0.001^{+++} compared to the Nic-Eth group. Data is shown as average. Statistical comparison between groups was done by ANOVA test and Tukey's post hoc test (the number of animals in each group was 10).*

7. The level of TNFα in liver tissue

Figure 7 shows the analysis of data obtained from measuring the amount of TNFα in different groups. The average values are shown in Table 1. According to the findings in Figure 7, TNFα was significantly increased in the treatment with Nic-Eth compared to the control group (P<0.001).

While the treatment of the groups with doses of 20 and 30 mg/kg significantly reduced this inflammatory factor (P<0.001). Treatment with crocin alone reduced the amount of this inflammatory factor compared to the control group (P<0.001).

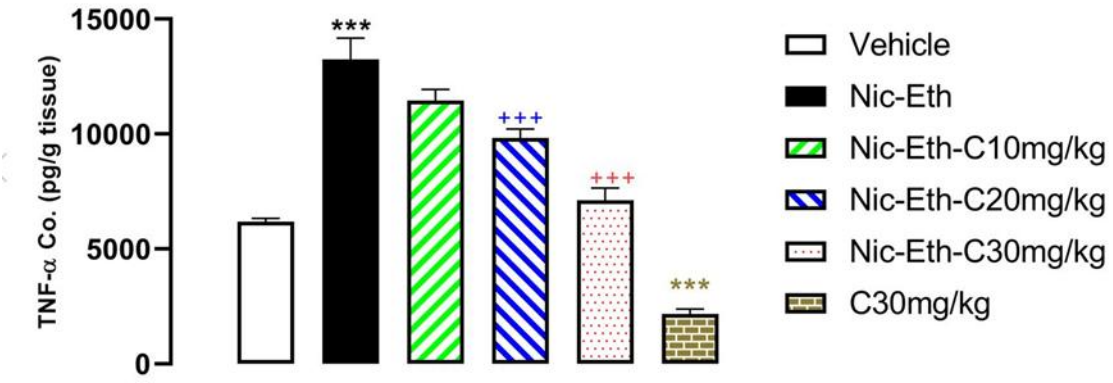


Figure 7. Evaluation of hepatic TNFα. p<0.05, p>0.01** and ***p>0.001 compared to the control group and p<0.05^{+}, p>0.01^{++} and p>0.001^{+++} compared to the Nic-Eth group. Data is shown as average. Statistical comparison between groups was done by ANOVA test and Tukey's post hoc test (the number of animals in each group was 10).*

Discussion

Nicotine and alcohol are among the most widely misused substances (1, 35). Overconsumption of one substance usually leads to excessive use of another in adolescents and adults. Even while each substance alone has a high danger to health, using them together can have negative synergistic effects (1).

Ethanol, a fast-acting compound due to its solubility in lipid and water, is absorbed from the stomach and intestines, rapidly diffuses into the blood circulation, affecting various body parts (33). Ethanol is rapidly oxidized to a more potent metabolite (acetaldehyde) than ethanol, resulting in direct and indirect adverse effects in humans (36, 37). Catalase, alcohol dehydrogenase, and the microsomal ethanol oxidation system are the primary pathways in ethanol toxicity that produce ROS, initiate apoptosis, lipid peroxidation, and induce oxidative stress in various parts of the body, including the liver and kidney (33, 38).

In addition, the risk of chronic kidney disease is high in smokers (39). Smoking is known to increase albumin excretion in the urine, decrease glomerular filtration, increase the risk of renal artery stenosis, and increase mortality in individuals with end-stage renal disease. The renal effects of nicotine are caused by the stimulation of the Cyclooxygenase-2 (COX-2) isoform. COX-2 isoforms promote glomerular inflammation, acute glomerulonephritis, and ureteral obstruction (40-42). Nicotine activates nicotinic acetylcholine receptors (αNAchR7), which negatively impact cell metabolism. This problem causes an increase in ROS generation and lipid peroxidation in several tissues, including the lung and liver (43). As a result, it is required to address complications induced by ethanol and nicotine in the liver and kidney.

Today, natural medicines are utilized to treat a variety of disorders because they are seen to be beneficial, safe, and effective (44). Therefore, in the present study, we decided to investigate the

effect of crocin on kidney and liver functional disorders caused by the combined consumption of nicotine and ethanol in adolescent rats.

Saffron (*Crocus sativus L*, from the *Iridaceae* family) is cultivated extensively in Iran, India, and Greece. Chemical investigations have identified crocin, crostin, picrocrocin, and safranal as the most significant saffron components (45). Several research on crocin's pharmacological effects have proven its analgesic (46), anti-atherosclerotic (47), anti-tumor, anti-cancer (48), anti-platelet aggregation (49), and antioxidant capabilities (50, 51). Crocin also protected against gentamicin-induced nephrotoxicity and suppressed microglial activation, reducing inflammation-induced neurotoxicity in mice (52, 53).

Our study found that crocin can improve the oxidant/antioxidant imbalance caused by ethanol and nicotine consumption in liver and kidney tissue. Groups treated with two doses of 20 and 30 30 mg per kg crocin showed a significant decrease in MDA, lipid peroxidation product, which is a commonly used biomarker of oxidative stress, and nitrite in the liver, and nitrite in the kidney. The dose of 30 mg per kg showed the most significant decrease in MDA in the kidney. The study found that groups treated with 20 and 30 doses of crocin showed a significant increase in the activity of SOD and catalase enzymes, and an increase in total thiol groups in the liver compared to the nicotine-ethanol receiving group.

In accordance with our findings, Rezaei Khorasany and colleagues demonstrated in their own investigation that ethanol use significantly increases MDA levels in the kidney and liver. While Crocin, (20 mg/kg, 28 days), was able to both restore and minimize the harmful effects of ethanol on MDA levels (44). Also, A. Hussain demonstrated that Crocin therapy has protective benefits, as evidenced by a considerable increase in kidney function and a decrease in the frequency of oxidative stress indicators. Crocin in conjunction with doxorubicin, an anthracycline antibiotic with nephrotoxicity as a significant side effect, has induced a drop in MDA and an increase in SOD in kidney tissues compared to the group receiving doxorubicin (54). According to the findings of Akbari and colleagues, Crocin enhanced the reduced activity of the SOD and catalase enzymes in the liver caused by hepatic ischemia-reperfusion damage in rats (129). As a result, crocin, as a potent antioxidant and radical absorber, can reduce MDA, the main product of lipid peroxidation, and increase the activity of SOD and catalase.

According to the findings of the current study, the examined liver enzymes, including alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), increased considerably in the nicotine-ethanol treatment group compared to the control. While the treatment of nicotine-ethanol receiving group with crocin significantly reduced all of the above liver enzymes. ALT and AST are required to detect liver disease, with ALT being more specific to the liver. High levels of these enzymes, along with ALP, can suggest numerous liver illnesses, such as chronic liver disease, acute hepatitis, and biliary obstruction (55).

Rezaei Khorasani and colleagues found that AST and ALT levels increased after one month of ethanol administration. while, Crocin treatment in mice reduced the levels of these biochemical indicators (44). Furthermore, Salahshor and et al. findings, which are consistent with ours, revealed a significant increase in ALT, AST, and ALP enzymes in the morphine-receiving group compared to the saline group, which was significantly stabilized by Crocin administration (56). A. E. Barhoma et al. also achieved comparable results, when they evaluated the potential therapeutic impact of crocin on liver damage caused by thioacetamide in albino male rats, they observed a

significant reduction in liver enzymes due to Crocin therapy (57). In another study, Crocin was able to significantly reduce the mentioned liver enzymes in rats treated with nicotine (58). As a result, the present study not only confirms the hepatotoxic effects of nicotine and ethanol, but also highlights the promising role of crocin in reducing liver damage.

Various studies have studied the therapeutic effects of crocin on improving kidney function (59-61). In our study, we also examined the level of blood urea nitrogen (BUN) and creatinine in groups treated with nicotine-ethanol and crocin. BUN and creatinine are essential biomarkers for evaluating renal function and health. Both chemicals are waste products that the kidneys remove from the blood, and an increase in their levels may suggest renal impairment (62). BUN and creatinine levels were significantly higher in the nicotine-ethanol receiving group than in the control group. Crocin treatment at all three levels reduced BUN, and two doses of 20 and 30 lowered creatinine as compared to the nicotine-ethanol group. Nicotine intake has elevated creatinine, urea, and uric acid levels (63). Similar to the findings of our study, Samarkandian and colleagues demonstrated that increasing age causes renal weakness, characterized by a significant increase in creatinine and BUN levels, but crocin treatment can reduce BUN and creatinine levels compared to untreated elderly rats (64). Also, Yirijani and his colleagues have observed a significant decrease in creatinine and urea nitrogen in mice treated with crocin. While creatinine and urea nitrogen were increased due to the administration of gentamicin to this group of mice (60). Therefore, according to the results of the studies, we can hope that crocin, as herbal and natural medicine, can be used as a supplement to improve kidney function disturbed by nicotine and ethanol.

Nicotine and ethanol both have significant inflammatory effects. Nicotine can have two functions, functioning as an anti-inflammatory in some situations and increasing inflammation in others, especially when pathogens are present. On the other hand, ethanol mostly causes inflammation and tissue damage, particularly in the liver and digestive tract (32, 65, 66). According to the findings of the present study, TNFα significantly increased in the treatment with nicotine-ethanol compared to the control group. Various studies have reported the anti-inflammatory effect of crocin (67, 68). We have also observed in our study that the treatment of groups with doses of 20 and 30 mg/kg of crocin significantly reduced TNFα in the liver tissue. Gedik and his colleagues observed a significant decrease in infiltration of inflammatory cells and vascular congestion in liver sections and intracytoplasmic vacuolation in liver cells after treatment with crocin (67). Aged mice showed impaired regulation of oxidative stress and inflammation in the kidneys, but treatment with crocin significantly decreased the expression of interleukin 6 (IL-6), TNFα, and interleukin 1 beta (IL-1β) (64). Also, Yirijani et al. have shown that crocin decreased TNFα in a dose-dependent manner (59). These findings indicate that crocin may serve as a useful therapeutic option to reduce the inflammatory effects of nicotine and ethanol and highlight its therapeutic potential in addressing inflammation-related disorders.

Conclusion

This study shows that in adolescents rats exposed to ethanol and nicotine, crocin has a substantial protective impact on kidneys and liver functions. The results show that crocin, with its potential anti-inflammatory and antioxidant characteristics, may effectively mitigate the damage to the organs induced by ethanol poisoning and nicotine by reducing inflammation and oxidative

stress. Improvements in biochemical indicators of liver and kidney function highlight crocin's capacity to protect these essential organs from damage brought on by chemicals. The potential of crocin as a therapeutic agent to prevent or mitigate organ damage is encouraging, given there is a growing trend of alcohol and nicotine use among adolescents. The goal of future studies should be to clarify the exact biological mechanisms behind crocin's protective effects and its potential for use in clinical practice.

Declaration

Data Availability Statement: The raw data supporting the conclusions of this study will be made available by the corresponding author upon reasonable request.

Conflict of interests: The authors declare that they have no competing interests and no conflict of interest regarding the publication of this manuscript.

Ethics Approval

All experimental protocols were approved by the Research Ethics Committee of Torbat Heydarieh University of Medical Sciences, Iran (Ethics Approval Code: IR.THUMS.REC.1403.027).

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