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ANALGESIC AND HEPATOPROTECTIVE EFFECTS OF *CHELIDONIUM MAJUS* L.

CHELIDONIUM MAJUS L.'UN ANALJEZİK VE HEPATOPROTEKTİF ETKİLERİ

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ABSTRACT

Water extract of Chelidonium majus L. (CM) was investigated for analgesic effect in mice and hepatoprotective effect in rats.

Analgesic activity of the extract was tested using tail-flick test. Mice were injected CM intraperitoneally (i.p.) in doses of 50 mg/kg, 100 mg/kg and 200 mg/kg. Pain tresholds were measured with tail-flick test before administration and at 30th, 90th and 150th minutes after treatment. Hepatoprotective activity of CM on carbon tetrachloride (CCl_4) induced acute liver toxicity was also studied. Rats were injected CM i.p. in doses of 100 mg/kg and 200 mg/kg.

CM had no analgesic activity at 50 and 100 mg/kg doses. However at 200 mg/kg dose, it produced higher analgesic activity than aspirin at 90th minute. At the 150th minute, its analgesic activity was equal to that of aspirin. CM (100 mg/kg and 200 mg/kg) showed no significant influence on the increased levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubine in CCl_4 treated

animals. Histopathological examination did not reveal any significant difference between CM (100 mg/kg) and CCl_4 groups.

The results of this study clearly indicate that C. majus. has analgesic activity at 200 mg/kg dose in mice. However C. majus. has no hepatoprotective effect against carbon tetrachloride-induced liver toxicity in rats.

Key words: *Chelidonium majus, Analgesic activity, Hepatoprotective activity, Papaveraceae.*

ÖZET

Chelidonium majus L. (CM) sulu ekstresinin farelerde analjezik, sıçanlarda hepetoprotektif etkisi araştırıldı.

Ekstrenin analjezik aktivitesi tail-flick testi kullanılarak yapıldı. Farelere CM ekstresi 50, 100, 200 mg/kg dozlarda intraperitonal (i.p.) yolla uygulandı. Ağrı eşiği, uygulamadan önce ve uygulamadan sonra 30., 90. ve 150. dakikalarda tail-flick testi ile ölçüldü.

CM'nin CCl_4 nedenli akut karaciğer toksisitesi üzerine hepatoprotektif aktivitesi de çalışıldı. CM, sıçanlara i.p. olarak 100 ve 200 mg/kg dozlarda uygulandı.

CM, 50 ve 100 mg/kg dozlarda analjezik aktivite göstermezken, 200 mg/kg dozda, 90. dakikada aspirinden daha yüksek analjezik aktivite göstermiştir. Analjezik aktivite 150. dakikada aspirine eşittir. CM (100 ve 200 mg/kg), CCl_4 enjekte edilmiş hayvanların yüksek olan aspartat aminotransferaz (AST), alanin aminotransferaz (ALT) ve bilirubin seviyesi üzerinde anlamlı bir etki oluşturmamıştır. Histopatolojik incelemeler, CM (100 mg/kg) ve CCl_4 grupları arasında anlamlı ölçüde belirgin bir farklılık olmadığını ortaya koymuştur.

Bu çalışmanın sonucunda *C. majus*'un farelerde 200 mg/kg dozda analjezik aktivite gösterdiği buna rağmen sıçanlarda karbon tetraklorür nedenli karaciğer toksisitesine karşı hepatoprotektif etki göstermediği açık olarak görülmektedir.

Anahtar Kelimeler : *Chelidonium majus, Analgesic aktivite, Hepatoprotektif aktivite, Papaveraceae.*

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INTRODUCTION

Chelidonium majus L. is a member of the Papaveraceae and it is growing in North Anatolia (1). *Chelidonium majus* has been used in folk medicine as diuretic, choleretic and hipnotic (2). It was used for skin conditions such as blister rashes, scabies and warts (3). The most effective alkaloid components of the plant (chelidonine, chelerythrine, coptisine, sanguinarine, berberine etc.) have spasmolytic, antiulcer, anti-inflammatory, antimicrobial, antiviral, antifungal and

antitumor activities and cytotoxic properties (4). The thiophosphoric acid chelidonine derivative from this plant called ukrain preparation, has been extracted by Nowicky (1991)(USA Patent No. 2, 670, 347) and has been tested with good malignotoxic activities in many cell lines (5). Öztürk et al. (1991) reported that *C. majus* is used for the treatment of liver diseases as an infusion in Anatolia (6).

Consequently, this work focuses on evaluation of the effectiveness of water extract of *C. majus* to normalize biochemical and histological parameters of CCl_4-induced liver injury. We also investigated the analgesic activity of *C. majus*. No reports were available on the evaluation of this plant for possible analgesic and hepatoprotective activity.

MATERIALS AND METHODS

Plant material.

Chelidonium majus L. was collected in 2003 from flowering plants near Çerkeş-Ankara (Turkey). Voucher specimens were kept in the Herbarium of Ankara University, Faculty of Pharmacy (AEF No. 22918). Taxonomic identity of the plant was confirmed by H. Duman a plant taxonomist in the Department of Biological Sciences, Faculty of Art and Science, Gazi University, Ankara, Turkey.

Preparation of extract.

Air-dried and powdered aerial parts of the plant were extracted with water. The water extract was prepared by macerating 20 g of plant powder in cold distilled water (300 mL) for one day. The macerate was evaporated and lyophillized (7,8). The yield was 20 % (w/w).

Animals.

The protocol for the study was approved by the Ethical Committee of Yuzuncu Yil University, Faculty of Medicine.

Male Swiss albino mice (22-30 g) and male Sprague-Dawley rats (150-210 g) were used in these experiments. The animals were obtained from the Animal House, Yuzuncu Yil University, Faculty of Medicine. All animals were housed in standard cages (48cm x 35cm x 22cm) at room temperature (22±2°C), with artificial light from 7.00 am to 7.00 pm, and provided with pelleted food (Van Animal Feed Factory) and water *ad libitum*.

Drugs and Chemicals.

The extract of *C. majus* dissolved in isotonic saline solution (0.9 % NaCl, w/v). The following agents were used; Carbon tetrachloride (Merck KgaA, 64271 Darmstadt, Germany), olive oil (Fluka Chemica, Steinheim, Germany) and Aspirin (Bayer, Turkey) was solubilized in isotonic saline solution.

Analgesic activity.

Analgesic response was assessed with a tail flick-apparatus (LSI Letica LE 7106, Spain) using a method initially described by D'Amour and Smith (1941) (9). Sixty albino mice were divided into six groups of ten animals each. The animals were gently immobilized by using a glove, and the radiant heat was focused on a blackened spot 1-2 cm from the tip of the tail. Beam intensity was adjusted to give a tail-flick latency of 5-8 sec in control animals. Measuring was terminated if the latency exceed the end of time (15 sec) to avoid tissue damage. In all experiments mice were tested twice, 30 min before drug administration in the baseline latency determined and 30, 90 and 150 min after drug administration. Three doses of the extract (CM-1: 50 mg/kg, CM-2: 100 mg/kg and CM-3: 200 mg/kg i.p.) were administered to three groups. Aspirin (100 mg/kg orally) and morphine hydrochloride (10 mg/kg subcutaneously) were used as reference standard (10,11). The group with isotonic saline served as the control.

Carbontetrachloride-induced hepatotoxicity.

The carbon tetrachloride (CCl_4) method described by Handa and Sharma (1990) was used for scheduling the dose regimen (12). Carbon tetrachloride (0.8 mL/kg i.p.) diluted in olive oil (1:1 dilution) was employed for inducing liver toxicity.

Fifty rats were distributed into five groups of ten animals each. Group I, which served as control, received isotonic saline solution (ISS) by intraperitoneal administration. Group II (olive oil control) received olive oil (0.8 mL/kg) i.p. once daily for 7 days. Group III (CCl_4: olive oil group) received 0.8 mL/kg CCl_4: olive oil (1:1) i.p. once daily for 7 days. Group IV received 0.8 mL/kg CCl_4: olive oil (1:1) and CM (100 mg/kg) i.p. simultenously for 7 days. Group V received 0.8 ml/kg i.p. CCl_4 and CM (200 mg/kg) i.p. once daily for seven days. All the animals were observed daily and any dead animals was subjected to post-mortem examination to find the cause of death. The rats were killed after 24 hours from the last examination done on the 7 th day. At the end of the treatment (8th day), blood samples were collected by direct cardiac puncture and the serum was used for the assay of marker enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin.

Body weights of the rats were measured at 8.30 am during the study. The percentage of daily changes in body weight (I) were calculated according to the formula (13):

The percentage of changes in body weight = 100 X ($weight_n$ - $weight_{initial}$) / $weight_{initial}$

$weight_{initial}$: Measurement of first day

$weight_n$: Measurement of 2,3,……10 days

Assesment of liver function.

The serum AST, ALT and total bilirubin concentrations were determined with a commercial kit (Roche) using a Roche Modular Autoanalyser.

Histopathological examination of the liver.

The livers of the experimental animals were fixed in 10 % neutral buffered-formalin prior to routine processing in paraffin-embedded blocks. Sections (4 μm thick) were cut and stained using Hematoxylin-Eosin (HE) stain.

Statistical analysis.

All data were represented as mean ± standard error mean (SEM). Analysis of variance (ANOVA) was used for the statistical analysis of data. Post-hoc LSD test (least significant difference test) was used for detemining significance. Results with $p<0.05$ were considered as statistically significant.

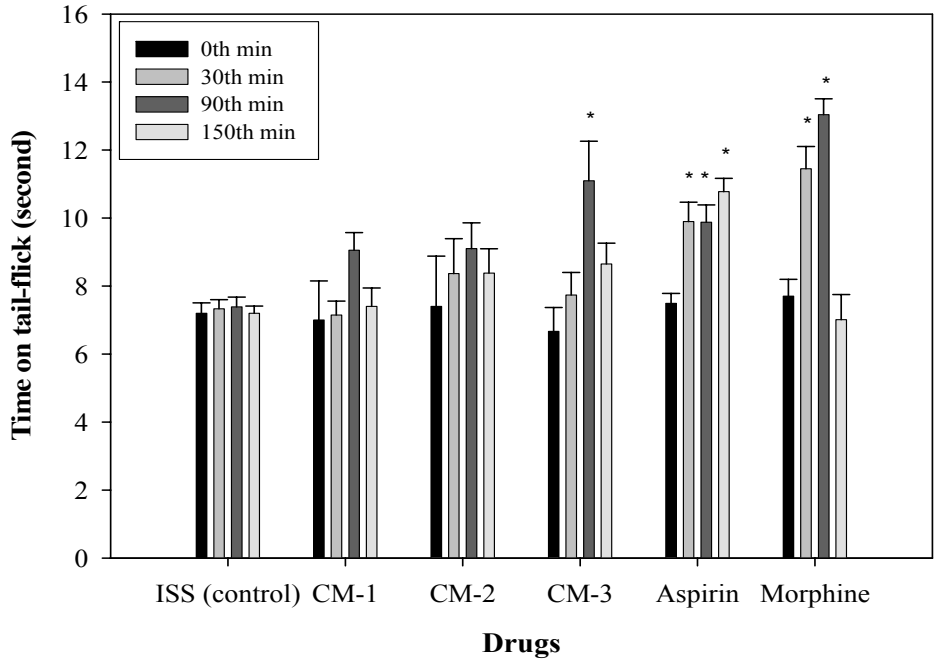
RESULTS

Follow-up of the working groups.

One rat was died in CM (100 mg/kg) group in 6^{th} day and two rats were died in CM (200 mg/kg) group in 4^{th} and 5^{th} days of hepatoprotective study. All other rats survived through the study. Physical conditions of CCl_4, CM (100 mg/kg) and CM (200 mg/kg) administered rats were worse than those of the controls showing decreased movement, vigility and food and water intake.

Analgesic activity.

Results of tail-flick test for analgesic activity were given in Graphic 1. Statistical analysis of the data revealed that aspirin and morphine caused significant analgesia at 30^{th}, and 90^{th} min compared to isotonic saline solution. Aspirine showed also statistically significant analgesic effect at 150^{th} minute after the administration. CM-3 application showed more analgesic effect compared to the controls at 90^{th} and 150^{th} minutes which was even more than aspirin at 90^{th} minute ($p<0.05$). CM-1 and CM-2 application did not result in analgesia compared to the control group ($p>0.05$).



CM-1: *C. majus* 50 mg/kg, **CM-2:** *C. majus* 100 mg/kg, **CM-3:** *C. majus* 200 mg/kg,
*: $p<0.05$ compared to control (ISS) group.
Graphic 1. Results of tail flick tests in *C. majus*, aspirin and ISS* groups.

Effect of CM on AST, ALT and total bilirubin levels.

The serum AST, ALT and total bilirubin levels were given in Table 1.

Table 1. Effect of *C. majus* on serum AST, ALT and bilirubin levels(mean ± SEM)

Groups	**AST** Serum (U/L)	**ALT** Serum (U/L)	**T. bilirubin** (mg/dL)
Control (ISS*)	137.3 ± 006.1	35.3 ± 003.5	0.09 ± 0.07
Control (olive oil)	127.8 ± 16.9	46.8 ± 3.4	0.04 ± 0.01
CCl_4	[ab] 1169.0 ± 306.6	[ab] 988.3 ± 266.7	0.18 ± 0.02
C. majus (100 mg/kg)	[ab] 761.5 ± 064.8	[ab] 434.8 ± 074.5	[ab] 0.46 ± 0.13
C. majus (200 mg/kg)	718.5 ± 182.4	[ab] 437.5 ± 150.7	[abc] 0.65 ± 0.14
F value	*4.116*	*2.835*	*10.175*
P value	*0.012*	*0.049*	*0.000*

***ISS:** Isotonic saline solution.
***P* values for Post-hoc Tukey's HSD test:**
b : $P<0.05$ for comparison with control group (ISS)
b : $P<0.05$ for comparison with control group (olive oil)
c : $P<0.05$ for comparison with CCl_4 group

Effect of CM on the rat body weight.

Percentage changes in weight were 1.7% in the ISS group, -1.72% in the olive oil group, -11.08% in the CCl_4 group, -8.96% in the CM-1 group and -10.06% in CM-2 group. The percentual daily body weight changes indicated that the CCl_4-treated group and CM groups had a high weight loss as compared to the control group.

Histopatological evaluation.

Histological damage was assessed with presence of ballooning degeneration, bridging necrosis and apoptosis. The liver histology in CCl_4 group rats showed extensive balooning degeneration, scanty apoptosis and bridging necrosis (Fig 1). The CM (100 mg/kg), CM (200 mg/kg) and the CCl_4 groups showed similar changes in liver. In histopathological examination ballooning degeneration, apoptotic bodies in hepatocytes, atipic nucleus and lymphocytic infiltration in portal areas were scarce (Fig. 2-5). In the control groups there were no histopathological changes in liver sections.

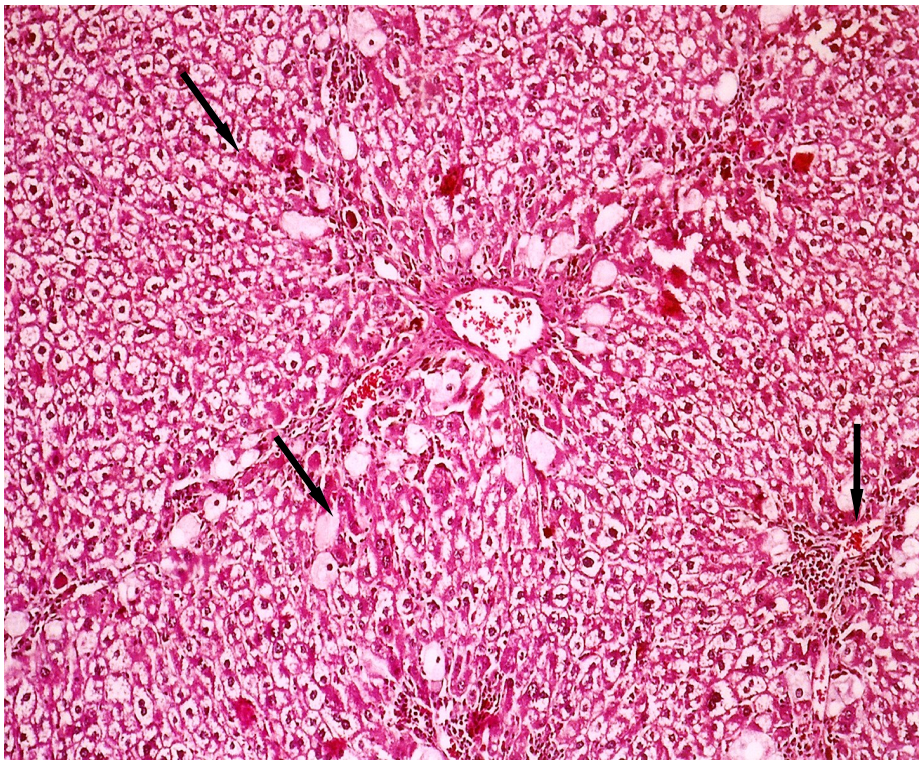


Figure 1. Numerous ballooned hepatocytes are seen in the liver of the CCl_4 group (Hematoxylin-eosin stain, original magnification, x100)

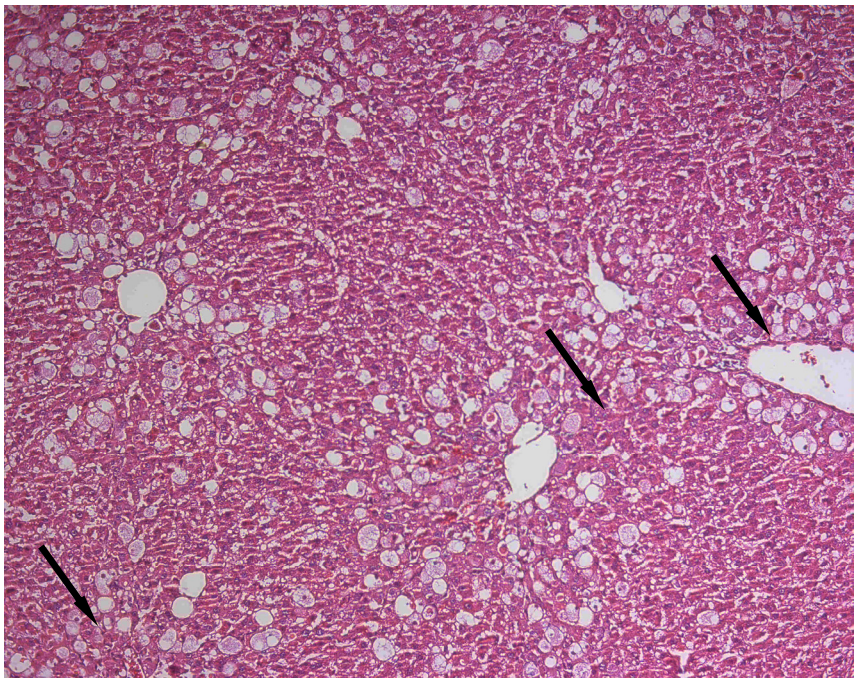


Figure 2. A few ballooned hepatocytes are seen in the liver of the CM (100 mg/kg) group (Hematoxylin-eosin stain, original magnification, x100)

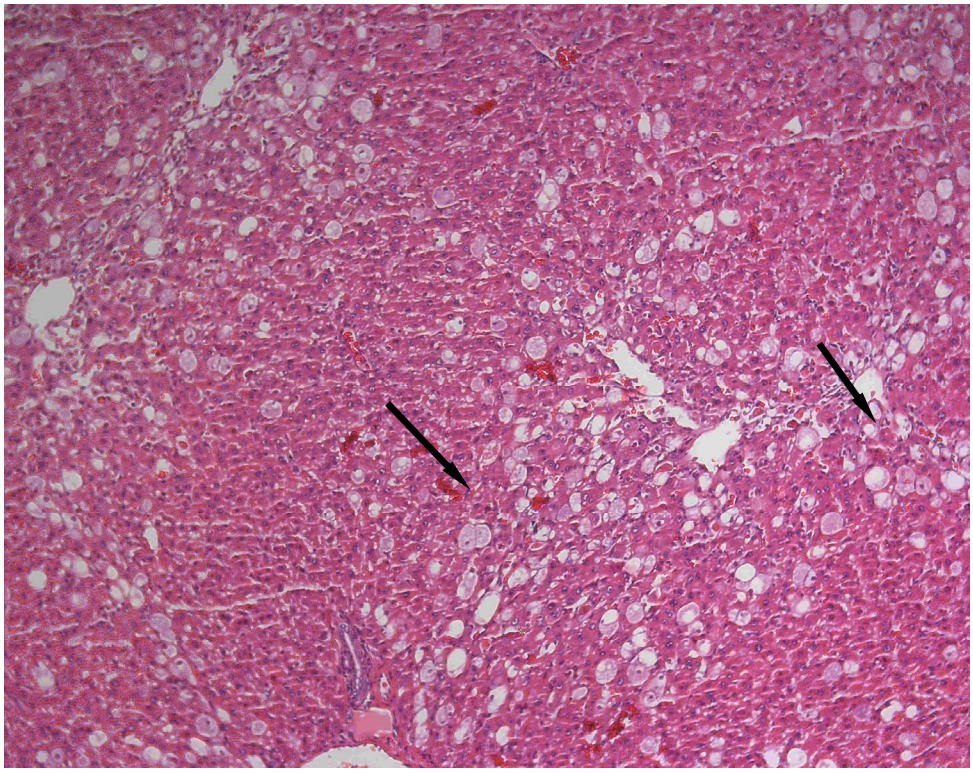


Figure 3. A few ballooned hepatocytes are seen in the liver of the CM (200 mg/kg) group (Hematoxylin-eosin stain, original magnification, x100)

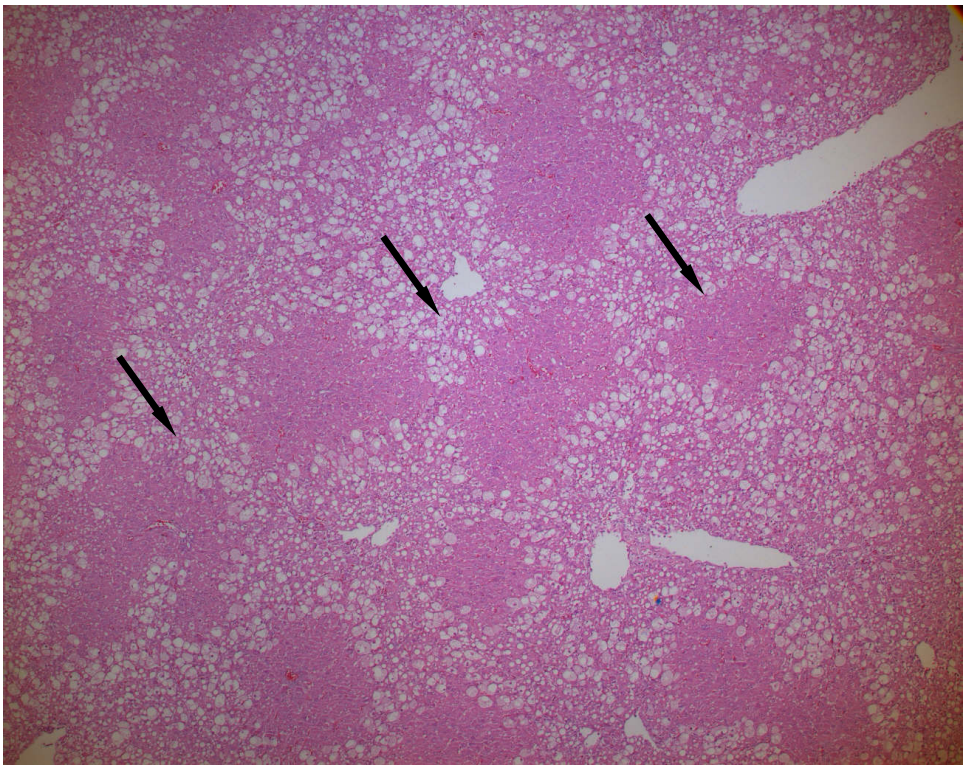


Figure 4. Bridging necrosis are seen in the liver of the CM (200 mg/kg) group (Hematoxylin-eosin stain, original magnification, x50)

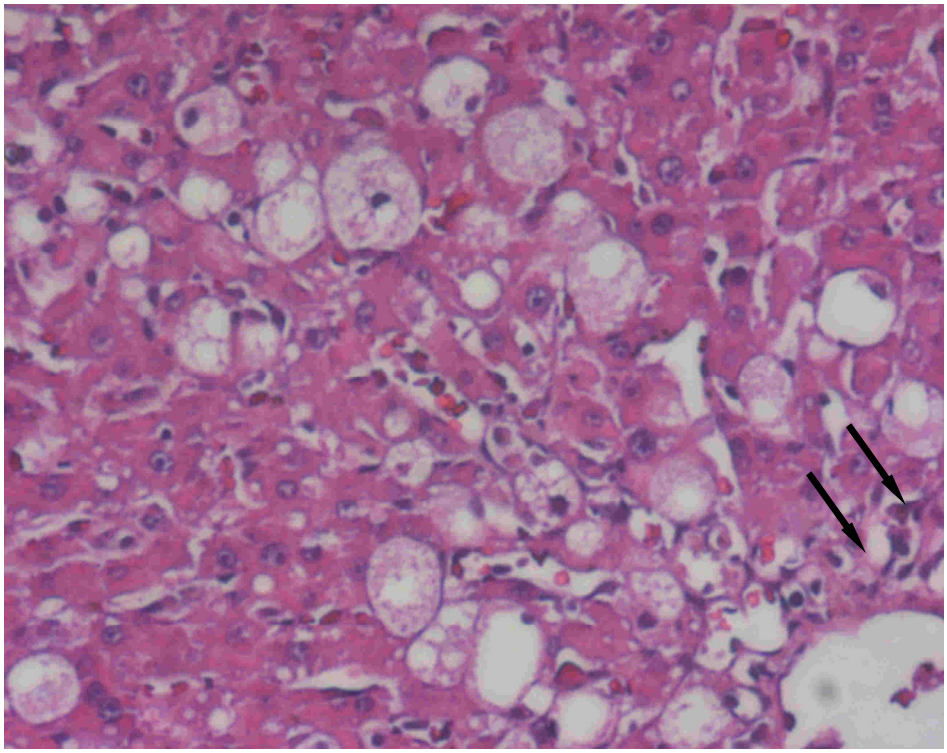


Figure 5. Rarely atipic nucleus and lymphocytic infiltration are seen in the liver of the CM (200 mg/kg) group (Hematoxylin-eosin stain, original magnification, x400)

DISCUSSION

In traditional and modern phytotherapy *Chelidonium majus* is used for the treatment of spastic discomfort of the bile ducts and gastrointestinal tract. It is used as cut herb, herb powder and dried extract for liquid and solid medicinal forms for oral use. *C. majus* have ben used in Turkish folk medicine as diuretic, choleretic and hipnotic as an infusion (6).

In England decoction of this plant or juice gargled have been used to relieve toothache (14). There are about a dozen OTC preparations with cholagogue and choleretic activities.

To obtund pain, powerful alkaloids with narcotic, analgesic and antibiotic properties are found within the *Papaveraceae* (15). Morphine-like properties associated with chelidonine and homochelidonine have been found in *C. majus*. However, the analgesic activity of water extract of *C. majus* (CM) has not been elucidated yet. Hence, the present study was carried out in an attempt to investigate CM for its analgesic activity using tail-flick test.

CM had no analgesic effect at doses of 50 mg/kg and 100 mg/kg. At a dose of 200 mg/kg it caused a significant analgesia at 90^{th} and 150^{th} minutes with was even more than aspirine at 90^{th} minute (Graphic 1).

We also studied the antihepatotoxic activity of CM on CCl_4- induced acute liver toxicity in rats. CM (100 mg/kg) did not cause any improvement in biochemical or histopathological aspects of acute liver toxicity induced by CCl_4. In CM group (100 mg/kg), serum bilirubin levels elevated more than controls (Table 1). In the same group (100 mg/kg) due to premature death of one animal, the histopathological and biochemical findings indicated that CM (100 mg/kg) had no beneficial effect in liver.

Two rats in the CM (200 mg/kg) group died during the experiment. The serum AST and ALT values of the remaining rats in the CM (200 mg/kg) group were lower compared to those in the CCl_4 and CM (100 mg/kg) groups. Histopathological findings in the CM (200 mg/kg) group show a liver damage though less severe than those in the CCl_4 group. So, we can say that CM (100 and 200 mg/kg) do not have hepatoprotective effects. The current results are supported by the acute liver toxicity due to CM in a 42 year-old female patient reported by Crijns et al. (16).

Greving et al. (1998) reported that *C. majus* is a rare reason for severe hepatotoxic reaction (17). Stickel et al. (2003) reported two cases of acute liver injury along with the intake of *C. majus*, a well known herbal remedy frequently used for irritable bowel syndrome (18). The present findings, supported by the findings of Crijins et al. (2002), Greving et al. (1998) and Stickel et

al.(2003) suggest that *C. majus* should not be used as hepatoprotective (15-17). We also think that use of *C. majus* as a herbal medicinal plant should be reconsidered.

As a result, water extract of *C. majus* (100 mg/kg) did not have any protective effect in acute liver toxicity. It was effective for analgesia at the dose of 200 mg/kg. However, analgesic activity should also be studied in other analgesy models namely writhing test and hot plate test. Further studies may help to reveal chemical composition of this extract and the phytochemical analysis may reveal the component(s) responsible for the analgesic effect.

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