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Use of *Arctium lappa* Extract Against Acetaminophen-Induced Hepatotoxicity in Rats

Attalla Farag El-Kott, PhD[1,2,*], Mashael Mohammed Bin-Meferij, PhD[3]

[1] *Deanship of Scientific Research, King Khalid University, Abha, Saudi Arabia*
[2] *Department of Zoology, Damanhour University, Damanhour, Egypt*
[3] *Department of Biology, Princess Nora Bint Abdul Rahman University, Riyadh, Saudi Arabia*

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ABSTRACT

Background: Severe destructive hepatic injuries can be induced by acetaminophen overdose and may lead to acute hepatic failure.
Objective: To investigate the ameliorative effects of *Arctium lappa* root extract on acetaminophen-induced hepatotoxicity.
Methods: Rats were divided into 4 groups: normal control group, *Arctium lappa* extract group, acetaminophen-injected group, and acetaminophen treated with *Arctium lappa* extract group.
Results: The treatment with *Arctium lappa* extract reduced serum alanine transaminase, aspartate aminotransferase, and alkaline phosphatase in the acetaminophen group when compared with the control group. DNA fragments in the acetaminophen-injected group were also significantly increased ($P < 0.05$). The comet assay revealed increased detaching tail length and DNA concentration during the hepatic toxicity in the acetaminophen group. The malondialdehyde content was inhibited by *Arctium lappa* treatment (12.97 ± 0.89 nmol/mg) when compared with the acetaminophen-treated-only group (12.97 ± 0.89 nmol/mg). Histopathologic examination revealed that acetaminophen administration produced hepatic cell necrosis, infiltrate of lymphocytes, and vacuolation that were associated with the acetaminophen-treated animal group, but the degree of acetaminophen-induced hepatotoxicity was mediated by treatment with *Arctium lappa* extract.
Conclusions: *Arctium lappa* can prevent most of the hepatic tissue damage caused by acetaminophen overdose in rats.

Introduction

At therapeutic doses, acetaminophen is a safe and effective analgesic and fever reducer. In fact, it is the most commonly used drug in the United States.[1] In 2008 alone, more than 24.6 billion doses were sold.[2] However, overdose of acetaminophen can cause severe liver injury. The first cases of acetaminophen hepatotoxicity were reported in 1966.[3] It is now the principal cause of acute liver failure in many Western countries.[4] The liver is the largest and a functionally vital organ of the body that participates crucially in metabolism, excretory and secretory processes, synthesis, and detoxification.[5] The liver is an important target of acetaminophen's toxicity, oxidative, and xenobiotic stress because of its distinctive metabolism and relationship to the gastrointestinal tract.[6]

The primary pathways for acetaminophen metabolism are glucuronidation and sulfation to nontoxic metabolites. Approximately 5%, however, is metabolized in the liver by the cytochrome P450 enzyme family to the toxic matter *N*-acetyl-*p*-benzoquinone imine. *N*-acetyl-*p*-benzoquinone imine combines covalently to sulfhydryl groups provided by glutathione. Following depletion of glutathione stores, *N*-acetyl-*p*-benzoquinone imine binds to cellular proteins in the hepatic tissues, leading to hepatic toxicity.[7] So, acetaminophen is regarded as a predictable hepatic toxin where biochemical signs of hepatic damage will become apparent within 24 to 48 hours after the time of overdose and produce a dose-related hepatic centrilobular necrosis.[8] The base dose of acetaminophen to cause hepatotoxicity is believed to be between 125 and 150 mg/kg. The threshold dose to cause hepatic toxicity is 10 to 15 g and 150 mg/kg acetaminophen for adults and children, respectively. Mechanisms of acetaminophen hepatic toxicity include production of a toxic metabolite, dysfunction of mitochondria, and innate immunity alteration.[9]

Experimental examinations strongly suggest antioxidant supplements as a promising therapeutic intervention for the

* Address correspondence to: Attalla Farag El-Kott, PhD, Deanship of Scientific Research, King Khalid University, Saudi Arabia
E-mail addresses: elkottaf@kku.edu.sa, elkottaf@yahoo.com (A.F. El-Kott).

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avoidance and treatment of liver disorders.[10] Therefore, there is growing interest in the evaluation of the possible role of antioxidant phytochemicals capable of preventing or protecting against stress of oxidative agents associated with liver disorders.

Arctium lappa Linné, commonly known as bardana or burdock, is a member of the Compositae (Asteraceae) family, and its carrot-like root is commonly cooked and eaten as a vegetable in Asia. *Arctium lappa*, which can be found worldwide, is used therapeutically as a depurative, diuretic, and digestive stimulant and for dermatologic conditions. The root of burdock has long been cultivated as a common plant for dietary use and folk medical uses.[11] *Arctium lappa* extract has become a promising and important beverage because of its therapeutic activity.[12] Many health benefits of burdock have been reported due to different compounds of bioactive secondary metabolites. These compounds include, among others, lignans and flavonoids, for which *Arctium lappa* is an important natural source.[13]

In addition, several investigators have demonstrated that *Arctium lappa* displays hepatic-protective properties,[12] antibacterial properties against gram-positive and negative bacteria,[14] and anti-inflammatory effects[15] that might be due to its free radical scavenging activity.[16]

Dietary consumption of antioxidants from plant materials has been associated with ignoble incidence of diseases due to decrease of stress oxidation. Thus the aim of our study was to determine the therapeutic role of burdock root extract on acetaminophen-induced hepatotoxicity in rats.

Materials and Methods

Materials

Dried burdock (*Arctium lappa* Linné) roots and acetaminophen were purchased from an area market and pharmacy in Damanhour, Egypt.

Animals

Male Sprague-Dawley rats (approximate weight, 200-250 g) were housed within the Animal Center of the College of Science, Damanhour University. They were kept for a minimum of 2 weeks under environmentally controlled conditions (25°C [± 1°C] with 55% [5%] humidity) and a 12-hour light/dark cycle was maintained with feeding on a commercial solid diet (24% protein) with free access to food and water.

Preparation of Arctium lappa crude water extract

One hundred grams burdock root (*Arctium lappa*) was ground into tiny parts and boiled with 1000 mL distilled water for 60 minutes. The extract was filtered, and also the residue was filtered when boiled once more. The filtrates were mixed well, placed into freeze-drying bottles, keep at –60°C, and transformed into powder with the freeze-dryer. The income was 24.67% (i.e., the purity of the extract). The crude water extract powder was dissolved in normal saline orally administered to rats, with an administration dose of 300 mg/mL saline/kilogram weight of the animals.[17]

Experimental design

Forty-eight rats were randomly assigned to 4 groups:

- Group 1, a control group that received the same volume of saline as the acetaminophen-only group,
- Group 2, a burdock group that received 300 mg/mL saline/kilogram,
- Group 3, an acetaminophen group that received a single dose of 800 mg/kg acetaminophen via oral administration, and
- Group 4, a treatment group that received burdock (300 mg/mL/kg) 12 hours before an acetaminophen injection.

Body and liver weights, blood samples, and liver specimens were collected 30 days after acetaminophen treatment.

Measurement of hepatocellular enzymes

Heparinized blood samples were taken by cardiac puncture under anesthesia at the end of the experiment. Aspartate transaminase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured using a standard clinical automatic instrument (Beckman, Brea, California). The results of AST, ALT, and ALP activity are expressed in international units per liter.

Examination of DNA fragmentation in hepatocytes

ELISA kit detection for necrobiosis (Boehringer Mannheim, Ingelheim, Germany) that quantitatively detects cytosolic histone-associated DNA fragments was used to assess DNA fragmentation in hepatic tissue homogenates. Briefly, the homogenized hepatic samples were collected at the end of the experiment. The cytosolic fraction was separated from liver tissue homogenates by centrifugation at 13,000 × g at 4°C for 20 minutes and used as antigen supply in a sandwich ELISA with a primary antihistone antibody coated to the microliter plate and a second anti-DNA antibody coupled to peroxidase. The %DNA fragmentation was computed in line with the equation

Single-cell gel electrophoresis (comet assay)

The liver tissues of each animals' group were removed and quickly kept at −80°C for comet assay. These specimens were homogenized in cooled buffer (pH 7.5) that contained 75 mM sodium chloride and 24 mM salt of disodium EDTA (pH 13) to get a 10% homogenate hepatic solution. A homogenizer was used, then the liver samples were kept on ice throughout and during homogenization. On cold slides, 6 μL homogenate hepatic solution was suspended on 0.5% low melting agars and sandwiched between a layer of 0.6% normal melting agars and a high layer of 0.5% low melting agars. The slides were kept on ice throughout the polymerization of every gel layer. When solidifying the 0.6% agars layer, the slides were immersed in a lysis solution (2.5 M NaCl, 1% sodium sarcosinate, 10 mM Tris-HCl, 1% Triton X-100 (Sigma-Aldrich, USA), 100 mM Na_2EDTA, and 10% DMSO) at 4°C. After 1 h, the slides were placed in electrophoresis buffer (1 mM Na_2EDTA, 0.3 M NaOH, pH 13) for 10 minutes to allow DNA to unwind. Electrophoresis was performed at 300 mA and 1 V/cm for 10 minutes. The slides were neutralized with pH 7.5 buffer solution of Tris-hydrogen chloride, and stained with ethidium bromide (20 mg/mL). Every slide was analyzed employing a Leitz Orthoplan epifluorescence microscope (Wetzlar, Hesse, Germany). One hundred cells were analyzed on every slide using the comet assay II automatic digital analysis system. The tail length (in millimeters) is the distance of DNA migration from the center of the body of the nucleus, and it is used to measure the extent of DNA injury. The tail moment is outlined because the product of the tail length and a fraction of the total DNA in the tail (tail length × % DNA in tail). Each tail length and tail intensity was measured automatically by the image analysis software.[18,19]

Table
Body and liver weights in experimental animal groups.

Animal group	Body weight (g) (mean ± SD)	Liver weight (g) (mean ± SD)
Control	278 ± 1	7.98 ± 0.27
Arctium lappa	276 ± 2	7.63 ± 0.96
Acetaminophen	280 ± 2	8.61 ± 1.03
Acetaminophen ± *Arctium lappa*	277 ± 4	8.01 ± 0.31

Hepatic lipid peroxidation

The thiobarbituric acid reactive substance levels as an index of malondialdehyde (MDA) production were measured via the method of Draper and Hadley.[20] Briefly, hepatic tissue (0.5 g) was homogenized employing a Potter-Elvejham homogenizer with 3 mL of 0.1 M cold phosphate buffer (pH 7.4). The homogenate of hepatic tissues was centrifuged at 300 × g for 10 minutes. Wherever 100 μL normal saline and 400 μL thiobarbituric acid-trichloroacetic acid mixture were mixed with 100 μL supernatant, this was followed by incubation for 30 minutes in a boiling water bath and then cooled at room temperature. Following centrifugation at 300 × gram for 10 minutes, 100 μL of 0.7% TBA was mixed with 100 μL supernatant in a cuvette and read at 535 nm. The thiobarbituric acid reactive substance concentrations of the samples were derived using 1.1.3.3 tetraethoxypropane as a standard and were expressed as nanomoles of MDA per milligram of tissue.

Histopathologic examination and quantification of necrotic cells

One lobe of the liver was removed at the end of the experiments and was cut into longitudinal sections 3 to 4 mm in thickness. Liver slices were then fixed in 10% buffered formalin and processed to embed in paraffin. Hematoxylin and eosin staining was performed in line with standard histologic procedures on 4-μm sections. Quantification of necrotic cells was performed by image analysis (Interactive Image Processing, Alcatel, Paris, France) under light microscopy. Ten representative areas from every section consisting of 5 periportal and 5 perivenous zones were examined. The area of every high-power field was maintained at 1.27 mm^2. Areas of hepatocyte necrosis were delineated using the image analysis software, and the percent area of every high-power field affected was determined.

Statistical analysis

All information is conferred as means (SD). Statistical analysis was performed using ANOVA. Variations between experimental animal groups were statistically significant at $P < 0.05$.

Results

Body and liver weights in the animal groups

The body and liver weights of animals injected with acetaminophen followed by treatment with *Arctium lappa* extract (**Table**) were not significantly affected.

Hepatocellular enzymes levels

The treatment of animals with acetaminophen resulted in significant increases ($P < 0.05$) in plasma ALT, AST, and ALP when compared with the control group, which is suggestive of severe liver injury (**Figures 1A, 1B,** and **1C**).

DNA fragments in experimental animal groups

The number of liver DNA fragments in the acetaminophen-treated group was also significantly increased ($P < 0.05$ vs control group) (**Figure 1D**). In our study, we found that administration of acetaminophen to rats resulted in a significant increase in liver DNA fragmentation ($P < 0.05$ vs control group), indicating that acetaminophen is capable of inducing hepatic cell apoptosis.

Comet assay (genomic single DNA fragmentation)

As shown in **Figures 2 and 3**, the comet assay revealed increased detaching tail length and DNA concentration during

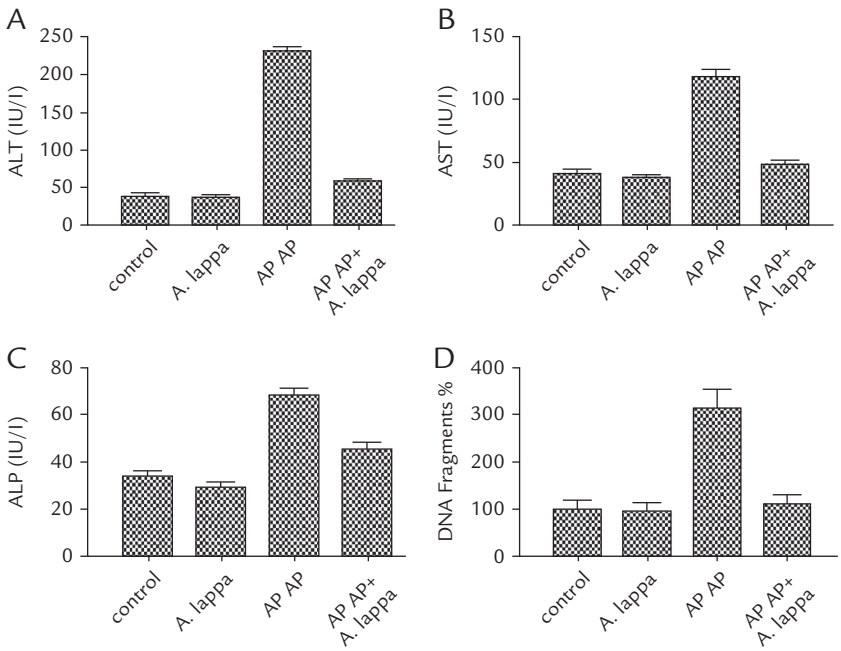

Figure 1. Effects of acetaminophen (APAP) on the alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) and DNA fragments in different experimental animals. A. lappa = *Arctium lappa* root extract.

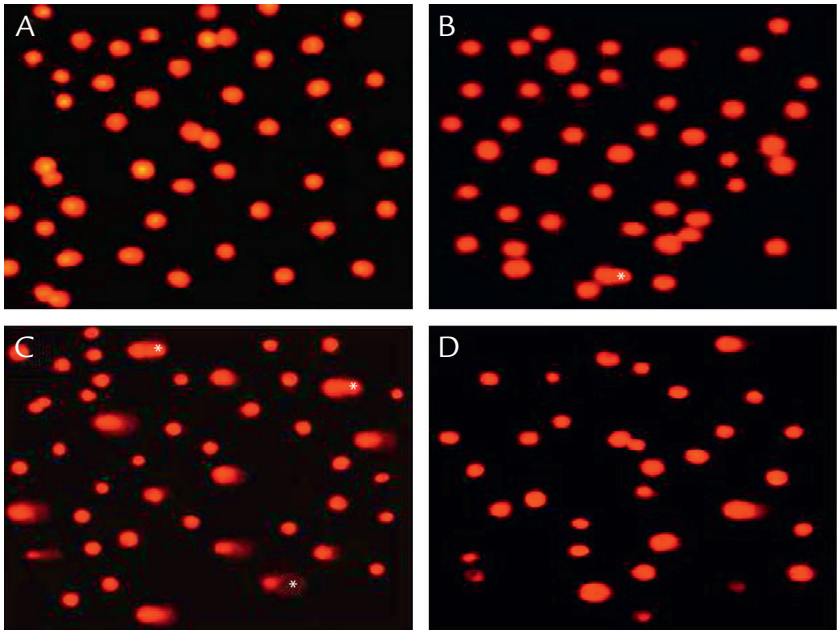

Figure 2. Comet assay of the hepatic tissues in (A) the control group, (B) the *Arctium lappa* group, (C) the acetaminophen group, and (D) the acetaminophen treated with *Arctium lappa* group. *Stretched hepatocyte with DNA damage, which becomes more abundant in the acetaminophen group.

the hepatotoxicity by acetaminophen in different experimental animal groups when compared with the control animals.

Hepatic lipid peroxidation

Lipid peroxidation has been reported to be related to acetaminophen-induced hepatotoxicity, so the content of MDA—the end product of the lipid peroxidation—was measured at the end of our experiment. Low levels of MDA were investigated in the control and *Arctium lappa* animal groups (9.81 [0.99] and 8.98 [1.09] nmol/mg tissue, respectively), but an increase was present in the acetaminophen-treated rats (18.98 [1.69] nmol/mg tissue). The MDA content was inhibited by *Arctium lappa* treatment (12.97 [0.89] nmol/mg tissue) (**Figure 4**).

Histopathologic evaluation

Acetaminophen administration produced hepatic cell necrosis in a zone around the terminal hepatic venules. The intense infiltrate of inflammatory lymphocytes and vacuolation were associated with the acetaminophen-treated animal group (**Figures 5C, 5D,** and **5E**). The *Arctium lappa* extract reduced the severity of all acetaminophen-induced hepatic responses, but the degree of reduction in acetaminophen-induced necrosis was incomplete: the dilated and congested central vein and some necrotic areas were present in the acetaminophen treated with *Arctium lappa* extract animal group (**Figure 5F**).

Discussion

In developing countries, 80% of indigenous populations depend on a local traditional medicine. Within European countries, medicinal herbs represent an important pharmaceutical market with annual sales of US $7,000,000.[21] Herbs have long been used as medical aids, and many currently available drugs are directly or indirectly derived from herbs. Recently, *Arctium lappa* tea derived from *Arctium lappa* has become an important and promising beverage because *Arctium lappa* exhibits free radical scavenging effects and has anti-inflammatory properties[22]; therefore, it can be used to avoid tissue damage by inducers of free radicals. Recently, the chemical components and clinical and pharmacologic applications of *Arctium lappa* Linnè were recorded.[23] There are 6 compounds of *Arctium lappa* Linnè seeds that can be isolated.[23] One of them is a new lignan called neoarctin B. The other 5 components were identified as lappaol F, arctiin, arctigenin, matairesinol, and daucosterol.[23] In traditional Chinese medicine for the treatment of common cold caused by heat and wind, the herbal drug *Arctium lappa* Linnè fruit is an often used. This drug includes many components, principally arctiin with a small amount of

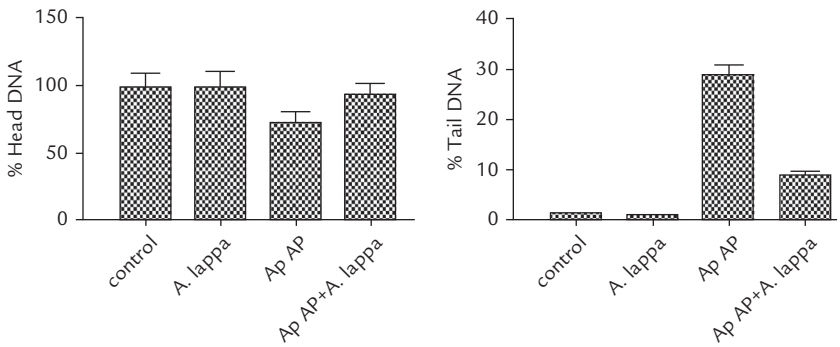

Figure 3. The head and tail DNA percentage in different animal groups. APAP = acetaminiophen; A. Lappa = *Arctium lappa* root extract.

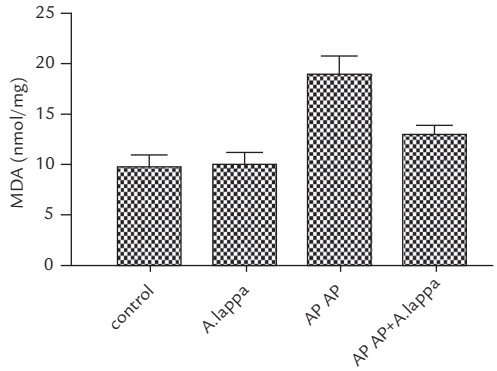

Figure 4. *Arctium lappa* (A. lappa) treatment inhibits hepatic lipid peroxidation (malondialdehyde production) after acetaminophen induction.

arctigenin.[24] In our study, the phytoprotective effects of *Arctium lappa* against acetaminophen-induced hepatocyte damage was investigated in rats.

Acetaminophen toxicity is one of the most widespread drug-induced side effects worldwide and damage to hepatic and renal tissues is a major complication with overdose of acetaminophen. The metabolites of acetaminophen created in hepatic tissue and tissues of other organs are likely to be the main contributors to its toxicity. The effects of acetaminophen are greatest in the necrotic centrilobular hepatocytes.[25] In our study, an oral single-dose administration of acetaminophen (800 mg/kg) was hepatotoxic in rats as shown by the significant increases in plasma ALT and AST activities as well as ALP concentration. The release of abnormal levels of hepatic enzymes in plasma is believed to be an indicator of hepatocyte injury.[26] The high concentrations of serum liver enzymes indicates hepatocyte damage because these enzymes are located in the cell cytoplasm and are released into the bloodstream following hepatic cell damage.[27] The association with lipid peroxidation and acetaminophen-induced hepatotoxicity has been reported in cases of acetaminophen overdose, and the end product of the lipid peroxidation is MDA, which was detected at the end of our experiment.

In our study, the comet assay was employed to assess the hepatotoxicity of the acetaminophen group, the acetaminophen treated with *Arctium lappa* group, and the other groups. The comet assay is used for the assessment of DNA damage in cells.[28] The different types of DNA damage and the DNA migration was detected by the comet assay. The level of double- and single-strand breaks and alkali-unstable sites is positively correlated with the extent of DNA migration. However, the crosslinks of DNA can be detected by the comet assay, as observed by the reduction of DNA migration when compared with the control cells.[29] According to our data, the decrease of the DNA migration was due to the antioxidant properties of the *Arctium lappa* extract, which would have decreased the level of intracellular free radicals with the consequent decrease in the occurrence of breaks due to oxidative stress causing damage in the DNA strand. The *Arctium lappa*

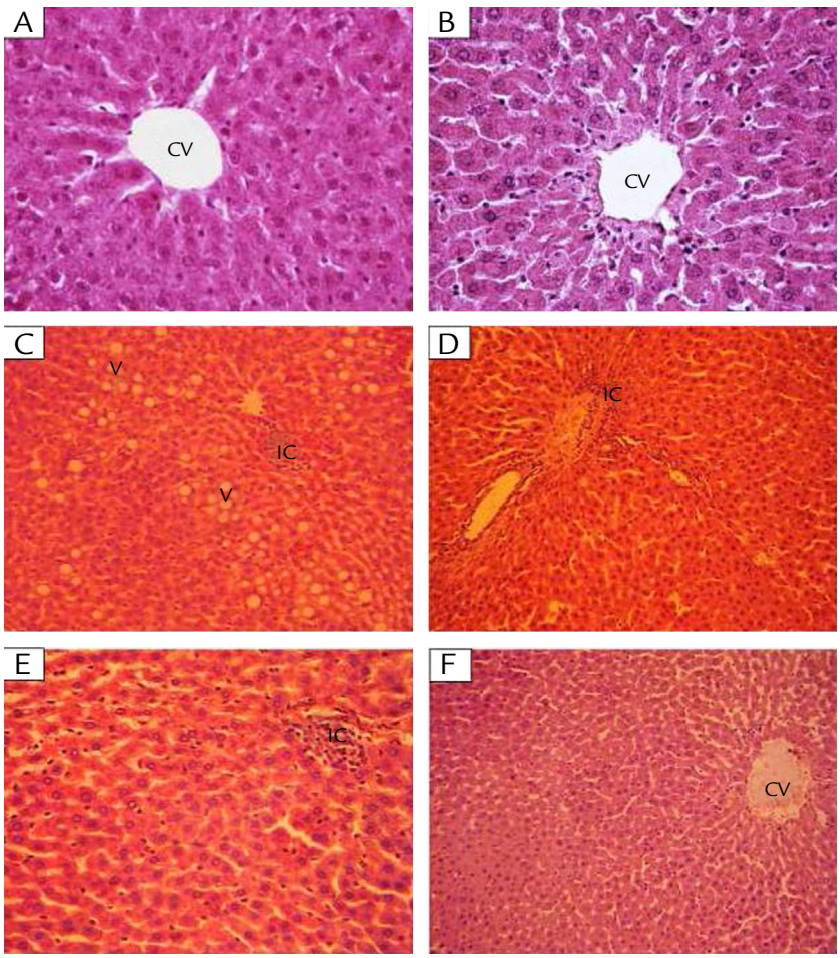

Figure 5. Hematoxylin-eosin stained hepatic sections (original magnification × 250) from different animal groups. (A and B) The hepatic tissues of control and *Arctium lappa* extract groups showing that the normal features of hepatic tissue and central vein (CV) structure. (C, D, and E) Sections from the acetaminophen-treated animal group showing the infiltration of inflammatory lymphocytes (IC), necrosis area, and fat vacuolation (V). (F) Section from acetaminophen-injected animals treated with *Arctium lappa* extract showing normal structure hepatocytes and CV.

decreased the DNA migration in a way that could result from a synergic effect of crosslinks between *Arctium lappa* extract components and acetaminophen. Our experiment has shown that the treatment of acetaminophen-induced hepatotoxicity with *Arctium lappa* extract retards DNA migration of the hepatocytes.

Hepatic injury is a common pathologic feature of many hepatic diseases. Therefore, treatment and prevention of hepatic injury is a key to clinical liver disease treatment. These results are consistent with the histopathologic findings in our acetaminophen group where the acetaminophen induced hepatocyte damage.[30] The histopathologic changes induced by acetaminophen overdose, including necrosis, infiltration of lymphocytes, and fatty liver, may be relieved by *Arctium lappa* extract. Binuclear hepatocytes were also present in our *Arctium lappa*-treated groups, which indicates the regeneration of hepatic cells (**Figures 5B and 5F**).

Lin et al[22] reported that *Arctium lappa* has free radical-scavenging properties. The inhibitory concentration $(IC)_{50}$ levels were 2.06 and 11.8 mg/mL *Arctium lappa* extract on superoxide-scavenging and hydroxyl radical activity, respectively. Perhaps the phytoprotective effect of *Arctium lappa* can be attributed to its free radical-scavenging activity and antioxidant effect, thus the removing of the injurious effects of toxic acetaminophen metabolites and inducing hepatocyte regeneration.

Conclusions

Our study showed that the *Arctium lappa* can mitigate most of the hepatic tissue damage caused by overdose of acetaminophen in rats. The phytoprotective activities of *Arctium lappa* demonstrated here and the fact that the tea of *Arctium lappa* has been used by humans make it a potential candidate for therapy of acetaminophen-induced hepatotoxicity in humans.

Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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