Short Communication

The Protective Effects of *Capparis spinosa* Aqueous Extract against Methotrexate in Male Albino Rats

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Abstract

Background: Methotrexate (MTX) concentrations in liver disorders can cause ischemia, requiring *Capparis spinosa* extract for antioxidant and free radical scavenging properties. **Objective:** The purpose of this investigation is to ascertain whether or not an aqueous extract of *C. spinosa* can lessen the cytotoxic effects of MTX in male albino rats. **Materials and Methods:** A total of 24 male albino rats were separated into four groups of six each. Oral normal saline (0.5 mL/kg BW) was administered to the placebo group. Group A received a weekly intraperitoneal injection of 20 mL/kg of MTX. The *C. spinosa* extract 250 mg/kg body weight group. The group was given MTX and *C. spinosa* 250 mg/kg by intraperitoneal (i.p.) injection weekly for 3 months. **Results:** Aspartate aminotransferase levels were significantly ($P < 0.05$) higher in the positive group compared to the control group. Alanine transaminase and alkaline phosphatase levels in the liver were also higher in participants who tested positive compared to those in the control group ($P < 0.05$). Compared to the control group, the treated group revealed no statistically significant changes in liver enzymes after receiving *C. spinosa* extract ($P < 0.05$). For the histological study, histological examination of the cross sections prepared from the liver of a rat injected with MTX as a single dose per week showed the presence of many histological lesions represented by the presence of degeneration and necrosis of the hepatocytes with rupturing of the wall of blood vessels, central veins, and hepatic veins, as well as the presence of infiltration of mononuclear inflammatory cells. **Conclusion:** The results of the present study focused that *C. spinosa* are promising hepatoprotective elements for improving defence mechanisms in the physiological and histological systems against oxidative stress under various circumstances.

Keywords: *C. spinosa*, hepato-protective effect, liver enzymes, methotrexat

Introduction

Since 1951, arthritis has been treated with methotrexate (MTX), a folic acid analog that has anti-proliferative, immunosuppressive, and anti-inflammatory action.[1,2] Rheumatoid arthritis (RA) and psoriatic arthritis (PsA) treatment is currently one of the most popular uses of MTX. These patients' quality of life has significantly improved thanks to MTX,[3,4] which has changed their natural history. RA has been treated with MTX since the 1980s, but the risk of hepatic adverse effects has been a major concern since then.[5,6] Many potential risk indicators for liver injury have been identified, but individual risk stratification is still not a part of the monitoring guidelines. The American College of Rheumatology and other prominent guidelines recommend monitoring liver enzymes in all RA patients using MTX every 8–12 weeks.[3] Liver enzymes are one of the most common screening tests used in rheumatology. Only a small number of studies have directly compared the effectiveness of different sets of monitoring recommendations. According to the results of two studies[8,9] evaluating the safety of a 12-week interval between tests, this period looks safe because no patients who were observed developed chronic increases in liver enzymes or other clinically relevant liver abnormalities. Traditional medicine frequently uses herbal medications, including medicinal plants and their

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bioactive ingredients.[7,8] The primary source of potential compounds with strong pharmacological effects is herbal plants.[9,10] The xerophytes deciduous perennial *Capparis spinosa* (Capparidaceae) can survive in a wide range of environments, from cold tundra to dry deserts. Folk medicine employs *C. spinosa* to cure a variety of liver conditions, diabetes, urinary incontinence, and rheumatic infections.[11,12] The chemical and bioactive components of *C. spinosa* have been widely studied, and they have been identified as having antioxidant and anti-inflammatory properties in a number of analytical studies.[13] The current work aimed to investigate the activity of *C. spinosa* aqueous extract against MTX in male albino rats.

Materials and Methods

Preparation of extracts

The alcoholic extract of *C. spinosa* leaves was prepared using a Soxhlet extractor device, taking 25 g of powder and adding 500 mL of absolute ethanol alcohol. The solvent was absorbed for 10 min, with a 20:1 ratio between the substance and solvent. The device was operated at boiling temperature for 2.5–3 h. The extracted solution was dried in an oven at 40–50°C and collected in a sterilized vial for use.[14]

Experimental animals

The research presented here was conducted at the University of Anbar, College of Education for Pure Sciences, and Department of Biology. Twenty-four adult male rats were supplied to a Baghdad veterinary hospital and pharmaceutical research laboratory. They were between 12 and 16 weeks old, with an average weight of 175–200 g. The animals were housed in clean, sterile environments, such as metal cages and glass bottles. During the whole study, participants had unrestricted access to food and drink. There were a total of 24 male albino rats used, and they were split into four groups of six:

- Negative group: which received orally normal saline (0.5) mL/kg BW.
- Positive assembly: 20 mg/kg of MTX was injected intraperitoneally in a single dose per week.[15]
- Extract group: It was given *C. spinosa* by a dose of 250 mg/kg body weight.

Treated group: which injected (intraperitoneal) by MTX and treated by *C. spinosa* 250 mg/kg weekly for 3 months.

Blood solution preparation

The blood was drawn from cardiac-punched rats and placed into test tubes containing EDTA. It was calculated how active the liver's enzymes were.

Histological study

The hearts of live rats were removed, fixed in 10% formalin, and dehydrated in progressively higher concentrations of ethanol. Tissue samples were dehydrated before being washed in two different types of xylene, impregnated in two different types of fluid paraffin wax, embedded, and blocked. Five micrometer-thick tissue slices were stained with hematoxylin and eosin.[16]

Statistical analysis

Statistical analysis Results were expressed using means and SE. Analysis of variance (ANOVA) was used to statistically evaluate the data to identify differences between the groups before and after the therapy using one-way ANOVA. The data were analyzed using SPSS (SPSS 2003, Statistical package for social sciences, IBM company, USA), and $P = 0.05$ was regarded as statistically substantial.

Ethical approval

The experimental protocol was approved in accordance with Order (179 on July 2, 2023) and issued by the Ethical Committee for the Care and Use of Laboratory Animals in the Department of Biology, College of Education for Pure Sciences, University of Anbar.

Results

Liver enzymes

Table 1 provided by you shows the results of liver function tests for four groups under study: the control group, the positive group, the extract group, and the treated group.

The numbers in the table are the levels of three liver enzymes: aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP). AST and ALT are released into the bloodstream when the liver is damaged. ALP is released into the bloodstream when the bile ducts become blocked.

Table 1: Activity of liver enzymes in the studied group

	AST (U/L)	ALT (U/L)	ALP (U/L)
Control group	19.83 ± 3.42	23.16 ± 6.17	53.83 ± 7.14
Positive group	105.28 ± 17.49*	89.74 ± 10.76*	193.53 ± 21.44*
Extract group	16.35 ± 4.94	20.58 ± 4.95	49.25 ± 9.13
Treated group	28.02 ± 7.83	25.94 ± 9.11	71.52 ± 13.92
P value	0.0001	0.0001	0.0001

Results of liver function tests show that rats in the positive group had significantly higher levels of AST, ALT, and ALP at the probability level ($P < 0.05$) compared to rats in the control group. This suggests that the rats in the positive group may have liver damage.

Rats in the extract group had significantly lower levels of AST, ALT, and ALP than rats in the positive group. This indicates that the extract may be useful in treating liver damage.

The results of the study did not record any statistically significant changes in liver enzymes resulting from *C. spinosa* extract ($P < 0.05$).

The current study's findings revealed that the MTX-treated groups had significantly higher serum levels of AST, ALP, and ALT than the control group and other groups.

Histological study

Control group

Microscopic analysis of the liver slices reveals the sinusoids to have normal diameters and widths, the central vein to be in its usual shape, and regular hepatocytes to be arranged in a radial pattern around the central vein, as shown in Figure 1.

Infected group

The liver transverse sections from this group were examined using histological methods, and the results can be seen in Figure 2. These histological lesions included the infiltration of mononuclear inflammatory cells, the degeneration and necrosis of the hepatocytes, and the rupture of the blood vessel walls, central veins, and hepatic veins.

Extract group

Microscopic analysis of the liver sections reveals the sinusoids to have normal diameters and widths, the central vein to be in its usual shape, and regular hepatocytes to be arranged in a radial pattern around the central vein, as shown in Figure 3.

Treated group

Hepatocytes were improved after the rats received MTX injections and *C. spinosa* treatment because they were nearly normal in terms of regularity and form and lacked the thickening of blood vessel walls shown in Figure 4. MTX-treated groups showed severe hepatic injury, as evidenced by degeneration, necrosis, and hemorrhage.

Discussion

An increased AST level may indicate muscle injury, liver disorder, or myocardial infarction. An increased ALP level indicates endogenous or exogenous obstruction of bile ducts caused by infiltration. Treatment with *C. spinosa* has a therapeutic effect on the liver, reducing damage and improving histological structure. Phenolic compounds in *C. spinosa* have antioxidant activity against liver damage. Hepatotoxicity is linked to drug metabolism, and increased secretion of liver enzymes during acute cell mortification may be due to liver cell wall damage or injury. The enlarged

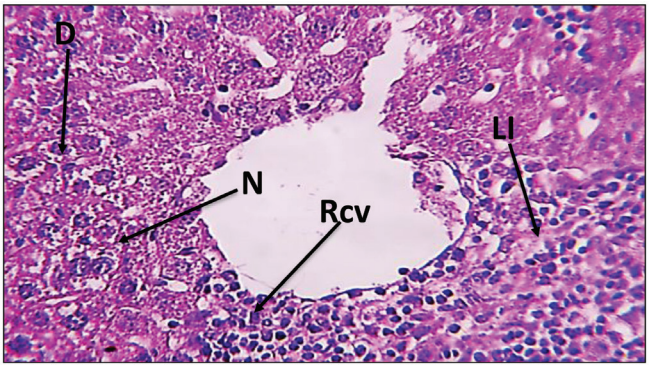

Figure 2: Liver section of MTX group showed rupture of central vein wall (Rcv), degeneration (D), and necrosis (N) of hepatocytes besides severe lymphocytes infiltration (LI) H&E ×400

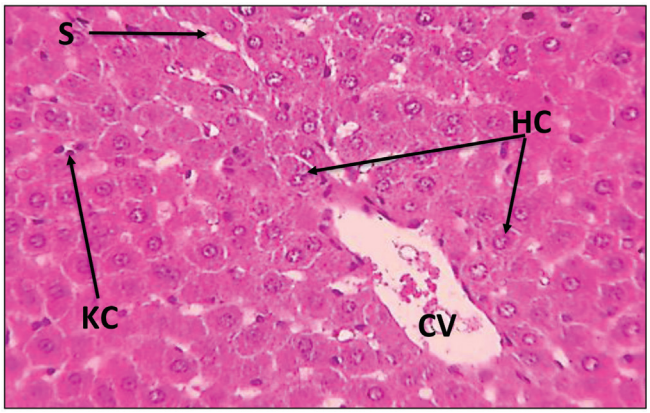

Figure 1: Liver section of control group displayed normal structure of central vein (CV), hepatocytes (HC), sinusoids(S), and Kupffer cells (KC) H&E ×400

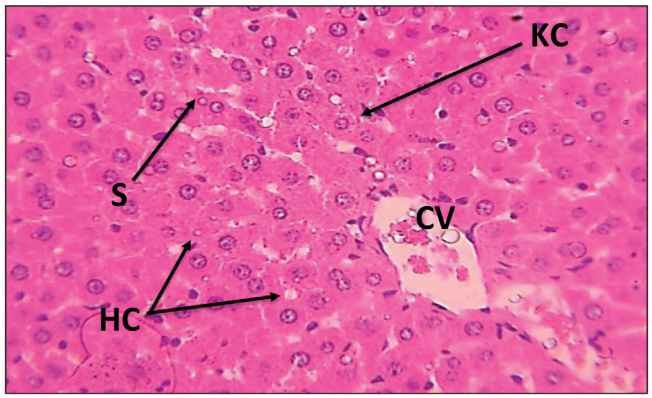

Figure 3: Liver section of extract group presented central vein (CV), hepatocytes (HC), sinusoids (S) in addition to Kupffer cells (KC) H&E ×400

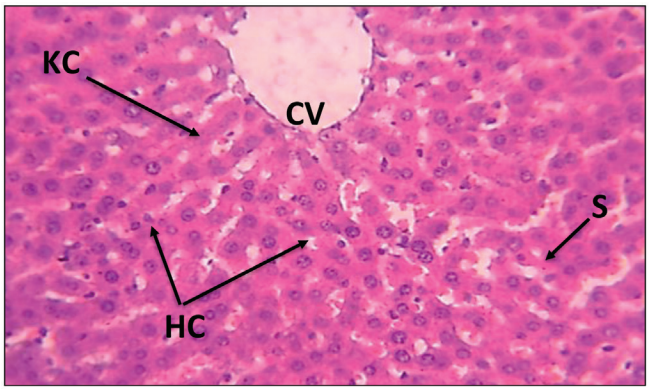

Figure 4: Liver section of MTX and extract group showed central vein (CV), hepatocytes (HC), and sinusoids (S) besides Kupffer cells (KC) H&E ×400

plasma level of enzymes in rats treated with MTX may be due to liver cell wall damage or injury, leaking enzymes into the blood circulation.

MTX is known to cause liver damage by increasing oxidative stress and weakening the antioxidant defense.[17] Lipid peroxidation and mitochondrial dysfunction brought on by MTX will eventually result in cell injury and damage to its membrane, which will cause the cell to lose its integrity and release the contents of the liver cell, particularly ALT and AST enzyme, into the bloodstream.[18,19] The administration of *C. spinosa* L. demonstrated significant improvement in liver enzymes (AST, ALT, and ALP) in the current investigation, supporting the role of *C. spinosa*. It is clear from the improvement in liver enzymes that *C. spinosa* L. possesses hepatoprotective properties. These protective effects could occur from stabilization of the plasma membrane, maintenance of the structural integrity of cells, and healing of MTX-induced hepatic tissue damage.[20]

Treatment with *C. spinosa* will improve health and lessen the impact of these lesions. The effect of *C. spinosa* alone was shown to improve the histological features of the liver tissues in comparison to the control group. It is common to compare MTX to folic acid while treating lymphoblastic leukemia of varying grades and other inflammatory illnesses such as RA.[21,22]

MTX is toxic to the liver because it inhibits the formation of catalase, glutathione, and superoxide dismutase, all of which serve as antioxidants. All told, this will lead to less s-adenosyl methionine being produced, which in turn leads to more reactive oxygen species being produced within cells. Damage to cells will result from an inverse association between a decline in antioxidant production and an increase in harmful reactive oxygen radicals. Several earlier investigations have confirmed that phenolic compounds extracted from *C. spinosa* L. have a hepatoprotective effect, which is relevant to the ability of *C. spinosa* in the current study. For example, From the leaves of *C. spinosa* L., p-methoxybenzoic acid (a phenolic component) was extracted by Gadgoli and Mishra.[23] It demonstrated considerable antihepatotoxic action against hepatotoxicity brought on by thioacetamide and galactosamine in isolated rat hepatocytes, as well as hepatotoxicity brought on by carbon tetrachloride and paracetamol in vivo. Bigoniya *et al.*[21] discovered that the flavonoid kaempferol, which was extracted from the *C. spinosa* L. plant, has a strong hepatoprotective effect on rat liver damage and CCl4-induced oxidative stress. The ability of kaempferol to restore normal levels of blood marker enzymes and improve the state of antioxidant defenses is connected with its ability to have hepatoprotective effects.[22] The results indicate that the extract under study can be used as an alternative and safe chemopreventive drug in the treatment of liver problems, which explains the function of *C. spinosa* in the current research.

Conclusion

We concluded that the extract of *C. spinosa* has therapeutic effects by inhibiting the toxic action, possibly due to its high content of antioxidants and other active substances. Our current study examined the effect of *C. spinosa* on the toxic effect caused by the administration of MTX.

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Conflicts of interest

There are no conflicts of interest.

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