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ORIGINAL ARTICLE

Ethanolic Extract of *Capparis decidua* Fruit Ameliorates Methotrexate-Induced Hepatotoxicity by Suppressing Oxidative Stress and Inflammation by Modulating Nuclear Factor-Kappa B Signaling Pathway

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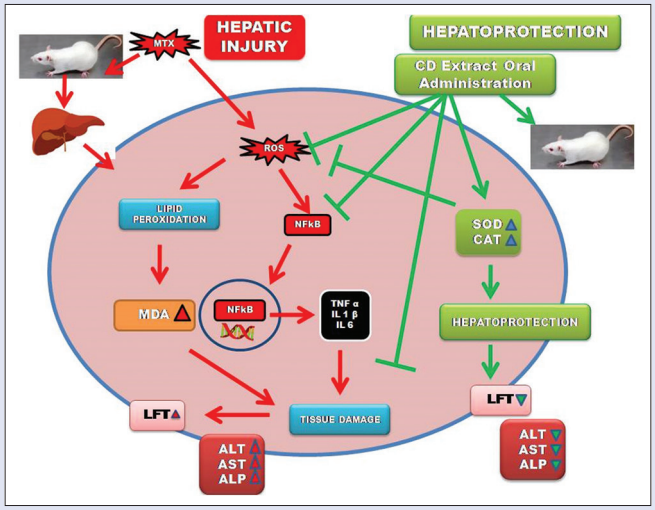
ABSTRACT

Background: Methotrexate is a stable derivative of aminopterin, a folate antimetabolite. Despite the widespread use of methotrexate, the clinical application of methotrexate is limited and restricted by the occurrence of hepatotoxicity. An important medicinal plant, *Capparis decidua* contains multiple pharmacological activities such as antioxidant, antiapoptotic, and anti-inflammatory effects. **Objectives:** This study investigated the protective effect of *C. decidua* against methotrexate-induced hepatotoxicity, focusing on its ability to attenuate oxidative stress and inflammatory nuclear factor-kappa B (NF-κB) signaling pathway which have not been studied earlier. **Materials and Methods:** Thirty-six female Wistar rats were randomly divided into six experimental groups. Rats were treated with *C. decidua* orally with doses of 250 mg/kg and 500 mg/kg for 14 consecutive days following a single dose of MTX injection (20 mg/kg, i. p). Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were assessed. Malondialdehyde (MDA) levels, superoxide dismutase (SOD), and catalase (CAT) were assessed in hepatic tissue. mRNA expression of, NF-κB, tumor necrosis factor-alpha (TNF α), interleukin-1beta (IL-1 β), and IL 6 was carried using real-time polymerase chain reaction in hepatic tissue. **Results:** Results showed that methotrexate administration significantly increased AST, ALT, and ALP levels. It has also increased MDA levels indicating lipid peroxidation and decreased antioxidants levels of SOD and CAT levels. Treatment with *C. decidua* significantly diminished the hepatotoxic effects of methotrexate which was seen in a significant decrease in AST, ALT, ALP, MDA levels, significant increase in SOD and CAT levels. Methotrexate provoked hepatic NF-κB phosphorylation and increased mRNA abundance of TNF-α, IL-1 β, and IL-6 expression. **Conclusion:** These findings suggest that *C. decidua* prevents methotrexate hepatotoxicity through suppression of NF-κB signaling pathway, attenuating oxidative damage, inflammation, and cell death.

Key words: Antioxidants, *Capparis decidua*, lipid peroxidation, methotrexate, NF-κB, oxidative stress

SUMMARY

- *Capparis decidua,* an important medicinal plant and it is used in the traditional system of medicine
- The study shows that the fruit of *Capparis decidua,* being a rich source of antioxidants confers protective effects against lipid peroxidation, oxidative stress, inflammation induced by methotrexate
- This could be due to the presence of alkaloids, flavonoids, terpenoids, glycosides present in the extract which act as natural antioxidants
- The study has thus explored the mechanism of hepatoprotective property of the fruit via the nuclear factor-kappa B signaling pathway.



A proposed schematic diagram illustrating the protective mechanism of *Capparis decidua* against MTX-induced Hepatotoxicity. MTX: methotrexate; ROS: reactive oxygen species; NF-κB - nuclear factor-kappa B; TNF α: Tumour necrosis factor alpha; IL-1β: interleukin-1beta; CD - *Capparis decidua*, ALT: Alkaline transaminase; AST: Aspartate Transaminase; ALP: Alkaline Phosphatase; MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase

Abbreviations used: MTX: Methotrexate; ROS: Reactive oxygen species; NF-κB: Nuclear factor-kappa B; TNF α: Tumour necrosis factor alpha IL-1 β: Interleukin-1 beta; CD: *Capparis decidua;* ALT: Alkaline transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; DPPH: 1,1-diphenyl-2-picryl-hydrazil; CCl_4: Carbon tetrachloride.

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INTRODUCTION

Liver is involved in the detoxification of drugs, which in turn may damage the liver.[1-3] Hepatotoxicity is liver damage caused by chemicals. Even certain medicinal agents when taken in overdose or in the therapeutic range may induce damage to the liver. Drug-induced hepatotoxicity is referred to as the most common causes of liver damage and are responsible for most of acute liver failure cases.[4] Drug-induced hepatotoxicity is found responsible for 5% of all admission in the hospital, 50% of all acute liver failure.[5] Methotrexate was initially used for the management of pediatric acute leukemia and later it has been extended to treat psoriasis and rheumatoid arthritis.[6-8] Methotrexate is a stable derivative of aminopterin, a folate antimetabolite, blocks the synthesis of purines and pyrimidines by inhibiting several enzymes involved in the pathway. Despite widespread use of methotrexate, clinical application of methotrexate is limited by the occurrence of hepatotoxicity.[9] Toxicity studies with methotrexate reported that oxidative stress has a very critical role in producing the most serious side effects which include hepatotoxicity.[10] Longer intracellular presence of methotrexate in the form of methotrexate polyglutamates is considered responsible for the mechanism of hepatotoxicity induced by methotrexate.[11] Methotrexate decreases folate levels as it has a high affinity to inhibit dihydrofolate reductase and also indirectly decreases DNA synthesis. Possible roles of inflammatory cytokines such as Tumour necrosis factor (TNF)-α mediating MTX-induced hepatotoxicity[12] have been studied recently. Recent studies have thrown its attention on the nuclear factor-kappa B (NF-κB) pathway to modulate hepatotoxicity-induced by methotrexate.[12]

Supplementation of substance which has good antioxidants would be considered appropriate method of approach towards hepatotoxicity of methotrexate. Traditional medicines are found to have hepatoprotective effects against xenobiotics.[13,14] Recently, medicinal plants are gaining great interest because of being a rich source of phytochemicals which may lead to the development of novel drugs.

Capparis decidua also commonly known as kair in Hindi, Caperberry in English is a very important medicinal plant, belonging to the family *Capparidaceae*.[15] It is reported to possess alkaloids, fatty acids, terpenoids, saccharides glycosides, flavonoids, volatile oils, and steroids that possess anti-inflammatory, odynolysis, antifungal, hepatoprotective, hypoglycemic, antioxidant, Anti hyperlipidemia, antistress and to improve memory.[16] Also, *C. decidua* is found to have hepatoprotective activity against hepatotoxicity induced by CCl_4.[17]

To our knowledge, no studies have evaluated the effects of *C. decidua* fruit ethanolic extract against methotrexate-induced hepatotoxicity and its role on NF-κB pathway. The aim of the present study is to explore and investigate the protective effect of *C. decidua* against methotrexate hepatotoxicity, focusing on its ability to ameliorate oxidative stress and modulate the inflammatory NF-κB signaling pathway.

MATERIALS AND METHODS

Chemicals

Methotrexate from Ipca Laboratories Pvt. Ltd, Mumbai and silymarin from Research-lab fine chem industries, Mumbai were obtained commercially. Other chemicals used for various other biochemical analyses were of analytical grade.

Preparation of *Capparis decidua* fruit extract

C. decidua fresh fruits was obtained, identified, and authenticated by Dr. Sunil Kumar, Department of Pharmacognosy, Siddha Central Research Institute (C18022101D). It was double washed with running water and then was shade dried. Using blender, the dried fruit was thoroughly ground to a fine powder. Ground powder of about 100 g was mixed with 500 ml of ethanol which was then placed in shaker for 24 h and it was subjected to boiling at 50°C with occasional stirring and it was filtered using filter paper and again subjected to boiling until concentrated extract was obtained and the yield was around 20 g of ethanolic extract and it was stored in a sterile container under-20°C.[18]

DPPH assay

Ethanolic extract of *C. decidua* fruit of different concentration was subjected to DPPH assay analysis as per the Shimada *et al.* method.[19] Ascorbic acid was used as standard and the absorbance of the standard and test was read at 517 nm. Different concentration of *C. decidua* fruit extract (100, 200, 300, 400, 500 µg/mL) was added to 1 ml 0.1 mM DPPH in methanol solution and incubated for about 30 min under dark condition. After the incubation period, absorbances of both test and standard samples were analyzed at 517 nm using UV-VIS Spectrophotometer, Elico SL 210. Percentage inhibition was calculated using the following equation

Percentage inhibition =

$$\frac{\text{Absorbance of the standard - Absorbance of the test}}{\text{Absorbance of the standard}} \times 100$$

Animals

Healthy female Wistar rats of age group 150 to180 days weighing about 180 ± 20 g were used for the study. The rats were maintained under standard condition– temperature (21 ± 2°C), under specific humidity (65 ± 5%), constant 12 h light and dark cycle. The rats were placed in polypropylene cages with paddy husk and were fed with a standard pellet diet and water. And the study protocol obtained approval from Institutional Animal Ethical Committee (Approval No: BRULAC/SDCH/SIMATS/IAEC/12-2019/037).

Experimental protocol

Thirty-six adult female Wistar albino rats weighing about 180–200 g body weight were randomly grouped into six groups with six rats in each of the group. Group I animals served as a control group which received normal saline for consecutive 14 days. Group II served as methotrexate group received intraperitoneal injection of methotrexate of dose 20 mg/kg body weight on the first day followed by an oral dose of normal saline for 14 days. Group III, Group IV, Group V received intraperitoneal injection of methotrexate of dose 20 mg/kg body weight on the first day followed by an oral dose of ethanolic extract of *C. decidua* 250 mg/kg body weight, *C. decidua* 500 mg/kg body weight, Silymarin in 0.5% CMC (positive control) 100 mg/kg body weight, respectively forconsecutive 14 days. Dose of methotrexate was selected based on the previous study.[20,21] Group VI served as the control and CD group which received an oral dose of *C. decidua* 500 mg/kg body weight for a period of 14 days. Dose of *C. decidua* and silymarin was fixed based on the previous study.[22,23] On the 15th day, sodium thiopentone (40 mg/kg body weight) was given as intraperitoneal injection to anesthetize the animals, and blood was collected through the retroorbital sinus and sera were separated, stored at – 80°C. The thoracic cavity was opened through the center using retractor clamp to expose the heart and the aorta was mounted on a stainless-steel cannula. While holding the heart steady with forceps, appropriate needle was used to collect 2–3 ml of blood sample from the heart preferably from the ventricle slowly to avoid collapsing of the heart. The needle was inserted directly into the protrusion of the left ventricle to extend straight up to 5 mm. Released the valve of the intravenous tube which is attached to the needle to allow slow and steady flow of around 20 ml/20 min of 0.9% saline solution. A cut was made in

the right atrium with sharp scissor to make sure that the solution flowed freely. The flow of saline without blood out of the atrium indicates that the perfusion is completed. The perfusion was stopped and the Liver tissues were immediately dissected out and used for further analysis. Liver homogenate prepared was used for subsequent biochemical assays.

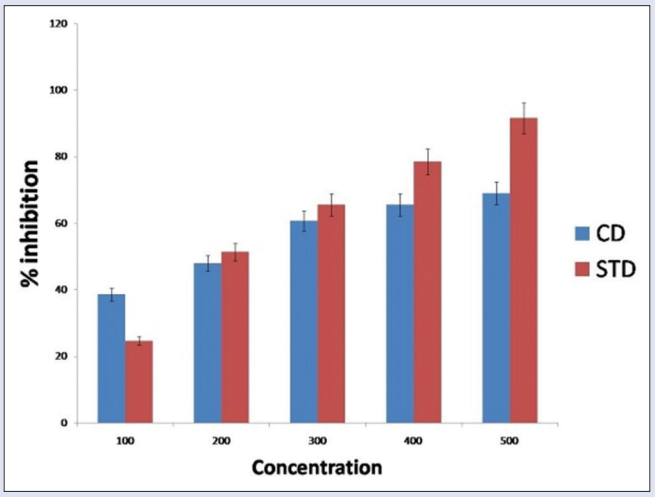

Figure 1: % Inhibition of 1,1-diphenyl-2-picryl-hydrazil by Ethanolic extract of *Capparis decidua*. Data are represented as means ± standard deviation Blue indicates standard and red colour indicates ethanolic extract of *Capparis decidua*

Biochemical assay

Liver function markers

Aspartate transaminase (AST), Alanine transaminase (ALT), and Alkaline phosphatase (ALP) were measured in serum as per manufacturer's instruction using kit commercially obtained from, Spinreact, Spain and values expressed as IU/L.

Lipid peroxidation

Malondialdehyde (MDA) content of the sample was measured to analyze the lipid peroxidation as per the method of Devasagayam and Tarachand, 1987[24] and lipid peroxidation was expressed in the form of nmoles of MDA formed/minute/mg of protein.

Antioxidant enzymes

The assessment of superoxide dismutase (SOD) and Catalase activity (CAT) was measured as per the method of Marklund and Marklund[25] and Sinha,[26] respectively, and the results were expressed as units/mg protein.

mRNA expression analysis

The gene expression analysis of NF-κB, TNF α, Interleukin-1 β (IL-1 β) and IL 6 was measured using real-time polymerase chain reaction (PCR)

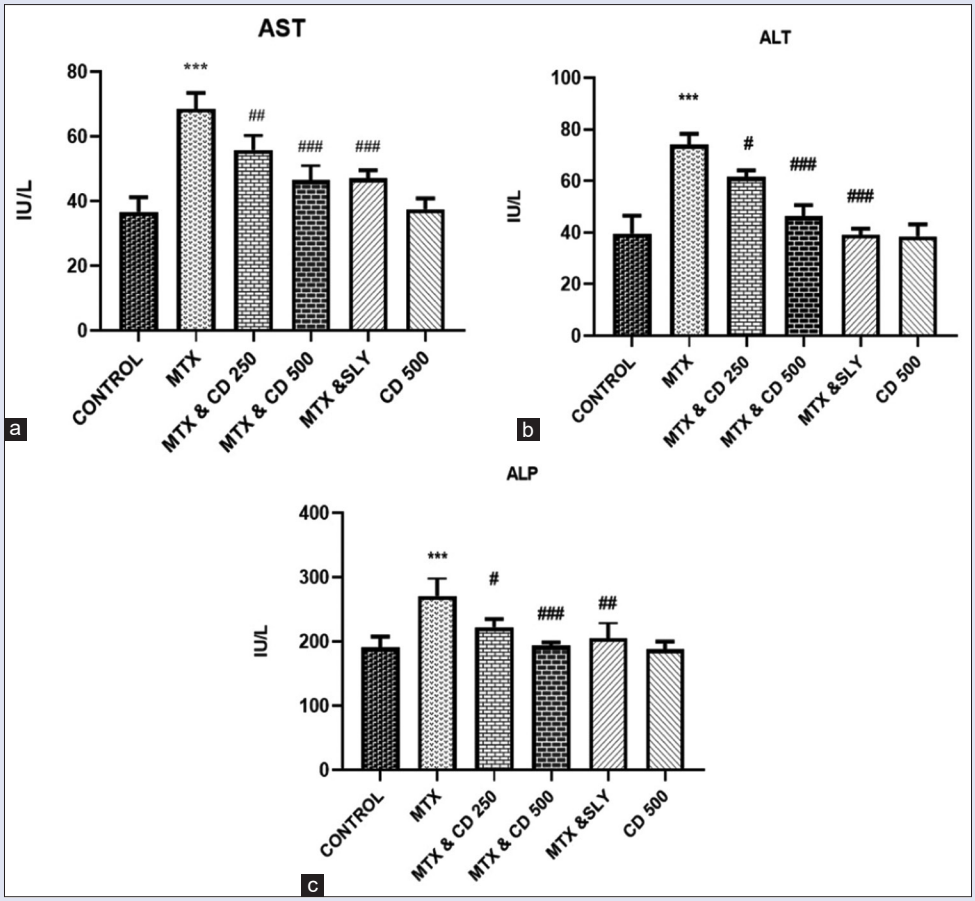

Figure 2: (a-c) Effect of treatment with CD on AST, ALT and ALP levels in MTX-induced hepatotoxicity. Data are represented as means ± standard deviation Data were analyzed by one-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons. Significant difference in comparison with the control group (*$P < 0.05$) (**$P < 0.01$) (***$P < 0.001$). #Significant difference in comparison with the MTX group (#$P < 0.05$) (##$P < 0.01$) (###$P < 0.001$). MTX: Methotrexate; CD: *Capparis decidua;* ALT: Alkaline transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase

as described previously.[27] Total RNA was extracted from liver tissue of control and treated animals per manufacturer's instructions using commercially available TRIR (total RNA isolation) kit procured from Invitrogen, USA. The RNA isolated was estimated spectrophotometrically and was expressed in micrograms (μg).

Complementary DNA was synthesized from 2 μg of total RNA using the reverse transcriptase kit from Eurogentec (Seraing, Belgium), as per the instructions in the manufacturer's protocol. The real-time PCR was performed using (Real-Time PCR Detection System). Relative quantification was obtained from the melt and amplification curves analysis [Table 1].

Analysis of histopathological changes

The liver tissues quickly isolated by fine dissection from all the groups were fixed in 10% neutral buffered formalin. The fixed liver tissue was passed into a serial ascending strength of ethanol and xylene, and was embedded in paraffin, and then 4–5-μm sections were cut using rotary microtome. The sections were completely dried and staining was performed with hematoxylin and eosin (H and E) stain and studied under a light microscope as per the method of Bancroft and Cook.[28]

Statistical analysis

Data were expressed as mean ± S. E. M and one-way ANOVA and Tukey's *post hoc* analysis multiple comparison tests were done to assess the significance using GraphPad Prism software version 5. A $P < 0.05$ was taken as significant.

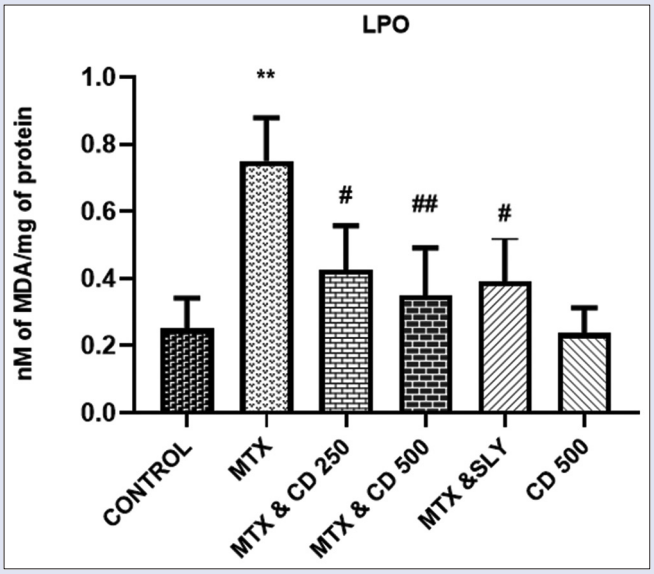

Figure 3: Effect of CD on LPO in liver tissue of methotrexate intoxicated rats. Data are represented as means ± standard deviation Data were analyzed by one-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons (MTX: Methotrexate; CD: *Capparis decidua)*. Significant difference in comparison with the control group (*$P < 0.05$) (**$P < 0.01$) (***$P < 0.001$). #Significant difference in comparison with the MTX group (#$P < 0.05$) (##$P < 0.01$) (###$P < 0.001$)

RESULTS

Antioxidant activity

The extract showed percentage inhibition activity of about 38.6% at 100 μg/mL and it was 48% at 200 μg/mL, 60.66% at 300 μg/mL, 65.53% at 400 μg/mL, 69% at 5000 μg/mL. Thus, it was seen that as concentration was increased, the % inhibition activity also increased and reached a maximum of 69% at 500 μg/mL concentration [Figure 1].

Effects of *Capparis decidua* on liver function

As shown in Figure 2, levels of liver function markers such as AST, ALT, ALP were significantly (all $P < 0.001$) elevated in methotrexate treated rats when compared to the control animals. Treatment with *C. decidua* administered at a dose of 250 mg/kg for a period of 14 days following a single IP injection of methotrexate significantly reduced MTX-induced increase in AST, ALT, and ALP levels ($P < 0.01$, $P < 0.05$, $P < 0.05$) Treatment with *C. decidua* at a dose of 500 mg/kg for a period of 14 days significantly reduced MTX-induced increase in all liver function parameters ($P < 0.001$). Administration of C. *decidua* 500 mg/kg alone to normal rats did not show any changes in the liver function markers [Figure 2a-c].

Effects of *Capparis decidua* on lipid peroxidation

In Group II rats, administration of methotrexate showed a significant ($P < 0.01$) increase in the LPO levels compared with control group. Treatment with *C. decidua* 250 mg/kg showed a significant ($P < 0.05$) decrease in LPO levels in the liver tissue homogenate. Groups IV and V rats, treated with *C. decidua* 500 mg/kg and Silymarin, produced a significant ($P < 0.01$ and $P < 0.05$) decline in the LPO levels compared to methotrexate-induced rats [Figure 3].

Effects of *Capparis decidua* on antioxidant enzymes

The effects of *C. decidua* on the SOD and CAT content are depicted in Figure 4. Administration of methotrexate has significantly reduced SOD and CAT levels compared to the normal animals (all $P < 0.01$). Treatment with *C. decidua* at the dose of 250 mg/kg for 14 e days following single-dose IP injection of methotrexate increased these levels of SOD and CAT levels when compared with the methotrexategroup and it was found to be significant statistically ($P < 0.05$). Treatment with *C. decidua* 500 mg/kg for a period of 14 e days following single-dose IP injection of methotrexate significantly increased the levels of SOD and CAT compared to the MTX group ($P < 0.01$ and $P < 0.001$). Administration of *C. decidua* 500 mg/kg for 14 days did not show any changes in the SOD and CAT [Figure 4 a and b].

Effect of *Capparis decidua* on nuclear factor-kappa B, tumor necrosis factor alpha, interleukin-1beta and interleukin-6 gene expression

As shown in Figure 5 a-d the administration of methotrexate revealed significant increase in the mRNA of NF-κB, TNF α, IL 6, and IL-1 β in liver tissue homogenate when compared to the control group, where as *C. decidua* 250 and 500 significantly caused downregulation of mRNA expression of NF-κB, TNF α, IL-1 β and IL 6when compared with the methotrexate treated rats ($P < 0.001$) [Figure 5a-d].

Effects of *Capparis decidua* on liver histopathology

Histological profile of animals in control group, i.e. Group I showed normal histology of liver with central vein, hepatocytes, and portal triads [Figure 6a]. Normal architecture of the liver cells was thus revealed in the control group. Methotrexate-induced rats revealed marked degree of necrosis with infiltration of polymorphonuclear cells, infiltration of inflammatory cells, fatty infiltration was present in the hepatocytes, and congestion of sinusoids [Figure 6b]. Treatment with *C. decidua* 250 mg/kg extract attenuated the effects of methotrexate compared to methotrexate treated rats group [Figure 6c]. However, treatment with *C. decidua* extract 500 mg/kg and silymarin in Group IV

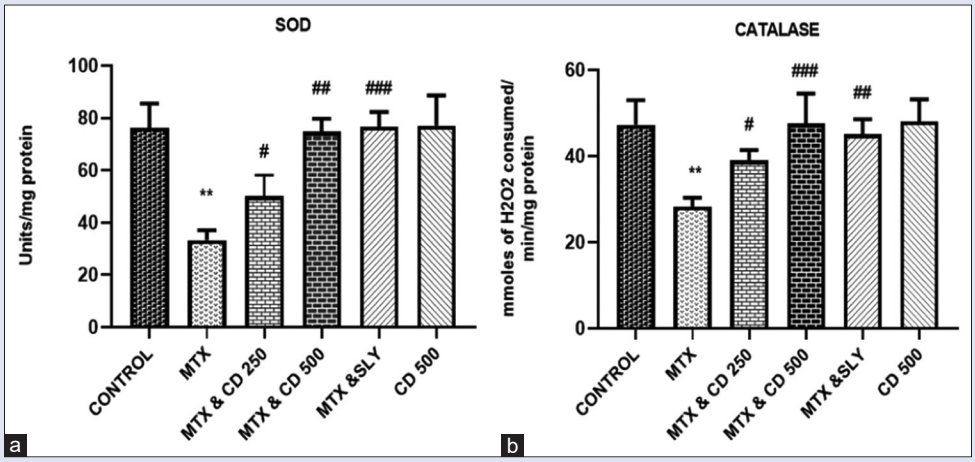

Figure 4: (a and b) Effect of CD on Catalase and Superoxide dismutase in liver tissue of methotrexate intoxicated rats. Data are represented as means ± standard deviation Data were analyzed by one-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons (MTX: Methotrexate; CD: *Capparis decidua*). Significant difference in comparison with the control group (*$P < 0.05$) (**$P < 0.01$) (***$P < 0.001$). #Significant difference in comparison with the MTX group (#$P < 0.05$) (##$P < 0.01$) (###$P < 0.001$)

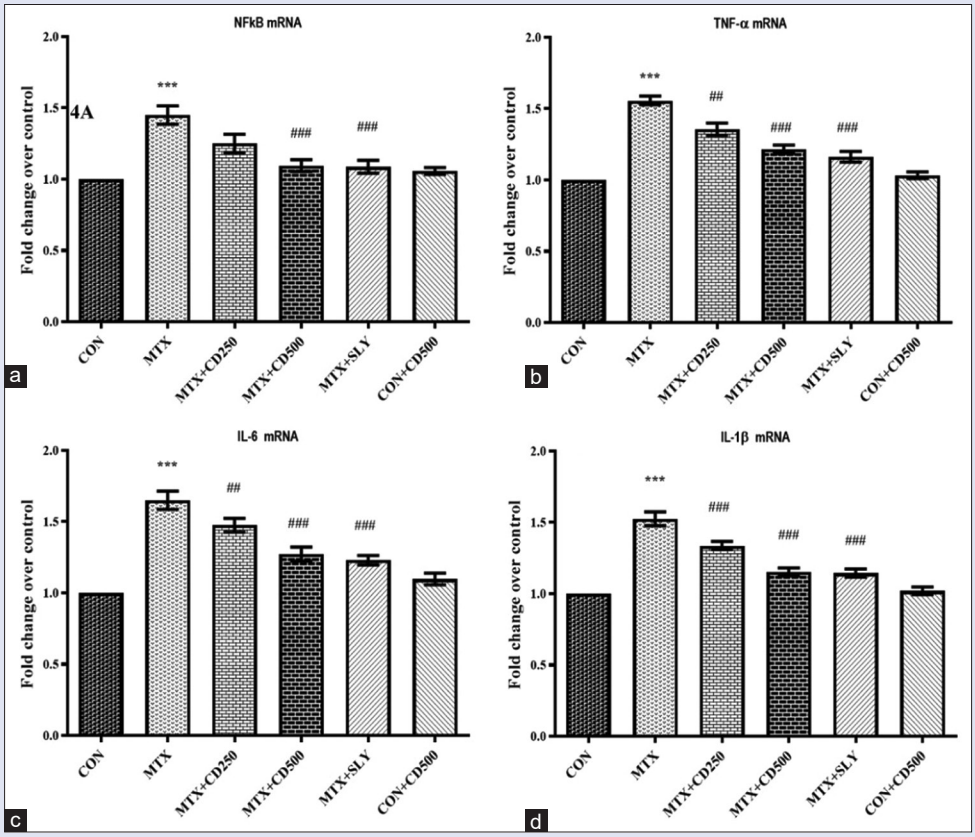

Figure 5: (a-d) Represents effect of CD on mRNA expression of NFkB, TNF alpha, IL-1 β and IL 6 in liver tissue of methotrexate intoxicated rats. Data are represented as means ± standard deviation Data were analyzed by one-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons. Significant difference in comparison with the control group ($P < 0.05$) (**$P < 0.01$) (***$P < 0.001$). #Significant difference in comparison with the MTX group (#$P < 0.05$) (##$P < 0.01$) (###$P < 0.001$). MTX: Methotrexate; ROS: Reactive oxygen species; NF-κB: Nuclear factor-kappa B; TNF α: Tumour necrosis factor alpha IL-1β: Interleukin-1beta; CD: *Capparis decidua;* ALT: Alkaline transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase

and V respectively showed regeneration of hepatocytes with normal architecture, with the absence of sinusoidal congestion and absence of fat deposits in the hepatocytes [Figure 6d and e]. Thus, Treatment with *C. decidua* restored the methotrexate-induced changes and revealed the beneficial effects of the extract in restoring the liver architecture which almost appeared nearly normal. Treatment with *C. decidua* 500 mg/kg did not alter the normal liver architecture of animals in group 6 [Figure 6f].

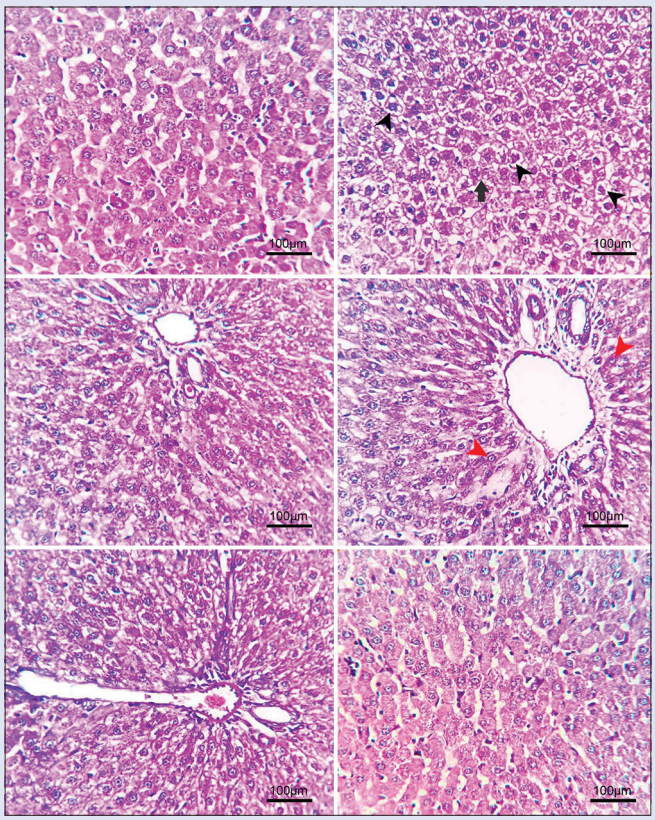

Figure 6: Histopathological observations (liver sections stained with H and E, magnification × 40) showing effects of CD on mtx induced hepatotoxicity changes in liver. (a) Control group; (b) MTX group showing fatty infilteration (Black arrow head), sinusoidal congestion (Bold arrow), inflammatory cell infilteration; (c) MTX + CD 250 group; (d) MTX + CD 500 group showing regeneration (Red arrow); (e) MTX + Sil group; (f) CD 500 alone group. MTX: Methotrexate; CD: *Capparis decidua*

DISCUSSION

Methotrexate is a widely used cytotoxic drug against several malignant conditions, inflammatory and autoimmune diseases.[29,30] The present study was done to assess the role of *C. decidua* extract against methotrexate-induced hepatotoxicity. The results of the study suggested that the administration of methotrexate elevated the levels of AST, ALT, and ALP. This was in agreement with the previous study by Tunali Akbay *et al.*[31,32] who also reported that methotrexate administration has provoked the notable elevation in serum ALT and AST. Elevation of ALT in the serum which is a cytosolic enzyme of the hepatocyte reflects a disturbance in the plasma membrane permeability leading to cell death. And liver necrosis is best indicated by the elevation of ALT levels.[33] Many previous studies have attempted to explain the mechanism behind hepatotoxicity induced by methotrexate. One such proposed mechanism by Ali *et al.* demonstrated that methotrexate induces hepatotoxicity by the generation of free radicals and by inducing oxidative damage.[34] Another study by Armagan *et al.* 2015,[35] has shown that methotrexate interference with antioxidant defense mechanisms causes an increase in lipid peroxidation. These studies have clearly reported the reactive oxygen species overproduction impairs liver function which was shown by elevation of liver function markers.[36]

Treatment with *C. decidua*, showed significant decline in AST, ALT, and ALP levels in methotrexate treated rats. Some studies have reported the administration of antioxidants attenuated methotrexate-induced hepatotoxicity.[34,36] The result could be attributed to the presence of flavonoids, glycosides, terpenes which are antioxidants and could be responsible for the hepatoprotective effect of the extract of *C. decidua* fruit. Similar studies have been carried out to study the hepatoprotective nature of *C. decidua* extract from stem which was found to be beneficial against carbon tetrachloride-induced hepatic damage[17] and reported that the presence of antioxidants was responsible for the hepatoprotective effect of *C. decidua*.

Lipid peroxidation is suggested to be an important contributing cause of methotrexate-mediated liver damage.[37] In the present study, methotrexate administered group showed elevated levels of MDA, which is considered as lipid peroxidation markers widely studied for oxidative stress. Hence, the present study suggests that lipid peroxidation, oxidative stress as a contributing factor for methotrexate-induced liver damage. This is in accordance with the various studies which also showed elevated MDA levels in methotrexate groups.[38,39] Administration of plant extract has significantly reduced the elevated markers of oxidative stress. *C. decidua* fruit contains many phytochemicals which act as antioxidants and could be responsible for alleviating oxidative stress.

Studies have reported that toxicity induced by methotrexate could be because of depletion of antioxidants such as CAT and SOD.[40] In the present study, methotrexate administration has reduced antioxidant such as CAT and superoxide levels whereas administration of *C. decidua* fruit extract has demonstrated the increased levels of CAT and superoxide levels suggesting that *C. decidua* extract reduces the methotrexate hepatotoxic effects by improving the antioxidant levels. Yadav *et al.* 1997 has also showed that *C. decidua* alters the levels of SOD and CAT and reduces the effect of oxidative stress.[41] Furthermore, previous studies have demonstrated that the other species of genus Capparis namely *Capparis spinosa* are found to possess protective effects against liver toxicity.[42]

The various previous studies showed that the extract of *C. decidua* possess potent antioxidant activity reducing different types of radicals such as DPPH.[43] Flavonoids have long been reported to possess hepatoprotective function.[44] It was observed in previous study that the presence of flavonoid in *Morus nigra* was responsible for the hepatoprotective effect of *Morus nigra* against methotrexate-induced hepatotoxicity.[45]

The hepatoprotective effect of the plant could be due to the presence of alkaloids, sterols, tannin, glycosides, flavonoids present in *C. decidua* fruit extract.[17,46] Also, the plant was reported to contain good sources of minerals such as iron, vitamin C which might contribute to the protective activity against liver damage as reported by another author.[17,47]

Inflammation is another important cause of hepatotoxicity induced by methotrexate.[48] Moreover, hence, the effect of *C. decidua* on the mRNA expression of inflammatory cytokines was evaluated. Sustained production of ROS activates the proinflammatory pathways. NF-κB signaling pathway was suggested t in gene regulation and pro-inflammatory cytokines activation including TNF-α.[49,50] NF-κB is a transcription factor present in the nucleus plays a role behind the pathology of hepatotoxicity induced by methotrexate. It is present in inactive state in the cytoplasm in combination with its inhibitor IkB alpha and IkB beta.[12] Oxidative stress leads to phosphorylation of IkBs leading to NF-κB release into the nucleus and NF-κB binds to the DNA and activates many inflammatory genes.[12]

The present study revealed that methotrexate administration induced an increase in mRNA of NF-κB which was in accordance with the previous reports.[51,52] Administration of *C. decidua* showed marked inhibition of mRNA of NF-κB inhibiting NF-κB pathway leading to the downregulation of many proinflammatory genes. Hence, to have a better understanding of the anti-inflammatory activity of *C. decidua*,

Table 1: Primer sequences for real-time polymerase chain reaction assay

Gene name	Forward primer (5′-3′)	Reverse primer (5′-3′)
NF-kB	CCTAGCTTTCTCTGAAC TGCAAA	GGGTCAGAGGCCAATAGAGA
TNF-α	TCAGTTCCATGGCCCAGAC	GTTGTCTTTGAGATCCATGCCATT
IL-1β	TGATGACGACCTGCTAGTGTG	TCCATTGAGGTGGAGAGCTT
IL-6	AGAGACTTCCAGCCAGTTGC	ACAGTGCATCATCGCTGTTC
GAPDH	TGGATTTGGACGCATTGGTC	TTTGCACTGGTACGTGTTGAT

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; NF-κB: Nuclear factor-kappa B; TNF-α: Tumour necrosis factor-alpha; IL-6: Interleukin-6; IL-1β: IL-1beta

we have evaluated the effects of mRNA expression of inflammatory genes in rats intoxicated with methotrexate. TNF α is plays a pivotal role in the production of inflammatory response causing damage to the liver. It also induces relaxation of other cytokines which in turn increases oxidative stress.[53,54] The present study has shown increased mRNA expression of TNF α, IL-1 β and IL 6 on methotrexate administration. This was supported by the previous study which also revealed the increased levels of mRNA TNF α, IL-1 β, and IL 6 on methotrexate administration.[12]

The results revealed that administration of *C. decidua* significantly downregulated the elevated inflammatory markers of mRNA levels of TNF α, IL-1 β, and IL 6 and this could be due to antioxidant property of the extract. Furthermore, the study revealed the downregulation of inflammation was nearly comparable to the reduction of inflammatory markers by administration of silymarin.

Histopathological findings also revealed abnormal, distorted fatty changes in the hepatocytes of methotrexate administered groups which were significantly reduced by administration of *C. decidua* extract along with methotrexate, and the extract was able to bring back the changes produced by methotrexate. This was also supported by biochemical changes.

Lack of investigation of the apoptosis pathway is one of the limitations of the study and so, therefore, future studies would be required to investigate the cellular mechanisms behind the hepatoprotective effect of *C. decidua*.

CONCLUSION

The result of the study indicates the *C. decidua* fruit showed good antioxidant activity. Furthermore, the present study indicates that methotrexate administration is associated with hepatotoxicity as a consequence of lipid peroxidation, oxidative stress, inflammation. Furthermore, the present study has demonstrated that *C. decidua,* being a rich source of antioxidants confers protective effects against lipid peroxidation, oxidative stress, inflammation induced by methotrexate. This could be due to the presence of alkaloids, flavonoids, terpenoids, and glycosides present in the extract which act as natural antioxidants and that could be responsible for increasing defense mechanism against free radical production induced by methotrexate administration. The extract thus has promising biological activity against methotrexate-induced hepatotoxicity acts by modulating NF-kB signaling pathway and by attenuating the lipid peroxidation, oxidative stress. However, still further detailed investigations are required to elucidate the exact phytochemicals involved in the protective role and to clarify the mechanism of action of the plant to combat hepatotoxicity of methotrexate administration.

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Conflicts of interest

There are no conflicts of interest.

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