

Ethanollic Extract of *Capparis decidua* Fruit Ameliorates Methotrexate-Induced Hepatotoxicity by Activating Nrf2/HO-1 and PPAR γ Mediated Pathways

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ABSTRACT

Introduction: Methotrexate, a folate antimetabolite, immunosuppressant and cytotoxic chemotherapeutic agents employed for malignant conditions. Continuous exposure of methotrexate produces adverse side effects especially liver toxicity. Recently, medicinal plants are gaining an importance owing to their phytoconstituents which are responsible for their medicinal value. *Capparis decidua* is one such medicinal plant widely used in the traditional system of medicine. No study has focused the mechanism of hepatoprotective property of *Capparis decidua* via Nrf2/HO-1 and PPAR γ signaling pathway. Hence the study was mainly focused on its ability to modulate Nrf2 and PPAR γ signaling in the methotrexate induced hepatotoxicity in Wistar rats. **Materials and Methods:** 30 female Wistar albino rats were divided into five groups. Following a single dose of methotrexate (20 mg/kg, intraperitoneally) injection, Wistar rats were orally administered with *Capparis decidua* at a doses of 250 mg/kg and 500mg/kg for 14 days. AST, ALT and ALP, antioxidants like GST, GPx, GR, were assessed in hepatic tissue. LPO, H₂O₂, OH radicals were assessed. mRNA expression of Nrf2, HO-1, PPAR γ was carried using real-time PCR in hepatic tissue. Nrf2, TNF- α , IL-1 β proteins were assessed using western blot. **Results:** Methotrexate administration significantly increased liver function marker, caused lipid peroxidation and decreased antioxidants levels. Treatment with *Capparis decidua* caused significant decrease in AST, ALT, ALP, levels and significant increase in GST, GPx, GR. Methotrexate decreased mRNA expression of Nrf2, HO-1, PPAR γ . *Capparis decidua* prevented methotrexate induced liver injury by upregulating Nrf2 and PPAR γ signaling pathway. *Capparis decidua* also suppressed inflammatory markers like TNF- α and IL-1 β proteins. **Conclusion:** *Capparis decidua* has thus attenuated methotrexate induced hepatotoxicity and this could be via up-regulation of Nrf2/HO-1 and PPAR γ pathways.

Key words: Antioxidant, *Capparis decidua*, Hepatotoxicity, Methotrexate, Nrf2, PPAR γ .

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INTRODUCTION

The vital organ of metabolism is liver and it is very important for maintaining homeostasis. Also it is essential for detoxification of drugs and they are easily exposed to xenobiotics and hence are

easily susceptible to the damage by these drugs.¹ Drug induced liver injury caused by medications and toxins, a common cause of acute liver failure is one of the most leading causes of non-approval and drug withdrawal



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from the market.^{2,3} The drug induced liver damage can progress to hepatocellular death, hepatitis and acute liver failure.⁴ Methotrexate is a folate antimetabolite, immunosuppressant and cytotoxic chemotherapeutic drug⁵ and it is a drug mainly employed in high doses for malignant conditions.⁶ It is an inhibitor of dihydrofolate reductase and it is used widely to treat various diseases like oncological disorders, rheumatological, inflammatory, dermatological conditions.⁷⁻⁹ Also it is a very important drug of choice for rheumatoid arthritis and psoriasis.¹⁰ However, Continuous exposure to methotrexate produces adverse side effects especially liver toxicity.¹¹ But the exact mechanism underlying the methotrexate induced hepatotoxicity has not been understood clearly and one of the proposed mechanisms for hepatotoxicity induced by methotrexate is oxidative stress and diminished levels of antioxidants.^{12,13} Methotrexate has been found to have a negative action on machinery of mitochondria which in turn produces ROS.^{14,15} The ROS in turn induces cellular damage causing peroxidation of lipids leading to cell death.¹⁶ Along with excess production of reactive oxygen species/ reactive nitrogen species, reduced antioxidant defence mechanism also promotes the development of hepatotoxicity.¹⁷ Hence the proper way of dealing with the oxidative stress and its attenuation would represent an appropriate method to prevent against hepatotoxicity induced by methotrexate.

Nrf2, which is Nuclear factor erythroid derived – 2 is basic leucine zipper (bZIP) protein that confers protection against oxidative stress by regulating the antioxidant defense proteins expression in the cell. Under normal circumstances, Nrf2 is anchored by Keap 1 (Kelch like ECH associated protein 1) in the cytoplasm.¹⁸ When exposed to oxidative damage, Nrf2 is released into the nucleus where it combines with ARE (antioxidant response element) in the DNA promoter gene by which it causes initiation of transcription of antioxidant genes which includes (HO) – 1 (Hemoxygenase), NQO-1 (NADPH quinoneoxidoreductase, SOD, catalase and other antioxidant genes.¹⁹ Thus activation of Nrf2 signalling pathway confers protection against oxidative stress induced by drugs and other agents.^{14,19,20} These findings indicate the very important role of Nrf2 in combating the drug toxicity mainly by increasing the cellular antioxidant defense mechanism.

Recently, PPAR γ (Peroxisome proliferator activated receptor gamma) has gained importance and proposed to play a very important role against liver injuries caused by drugs.^{14,21,22} The activation of PPAR γ produces anti-inflammatory effects and modulates the number of antioxidant gene expressions.²³ Thus activation

of PPAR γ would be one of the effective strategies to target oxidative stress induced by drugs. Since reactive oxygen species are implicated behind the pathogenesis of hepatotoxicity induced by methotrexate, supplementation of compounds or drugs rich in antioxidants can be considered one of the alternative methods to combat hepatotoxicity.

Recently, medicinal plants are gaining a significant role in pharmaceutical industries owing to their phytoconstituents which are responsible for their medicinal value in curing diseases.²⁴ *Capparis decidua* is one such medicinal plant widely used in the traditional system of medicine. It belongs to the family *Capparidaceae*. It is a xerophytic shrub found widely in North western parts of India. It is rich in phytochemicals such as stigmasterol, rutin, quercetin, spermidine, tocopherols, carotenoids, kaempferol, campesterol, it also possess many pharmacological properties such as antiviral, antibacterial, immunomodulatory, antimicrobial, anti-oxidative, anti-inflammatory properties, hepatoprotective property.²⁵⁻²⁷ Hepatoprotective effects of aqueous and methanolic extract of *Capparis decidua* stem against CCl₄ induced hepatotoxicity have been studied previously.²⁷ But there are no studies of ethanolic extract of *Capparis decidua* fruit against methotrexate induced hepatotoxicity. Also no study has focused the mechanism of hepatoprotective property via Nrf2/HO-1 signaling pathway. Hence the aim of the current study is to elucidate the protective effect of *Capparis decidua* fruit extract and to investigate the underlying action and the study was mainly focused on its ability to modulate Nrf2 and PPAR γ signaling in the methotrexate induced hepatotoxicity in Wistar rats.

MATERIALS AND METHODS

Chemicals

Methotrexate were purchased commercially belonging to Ipca Laboratories Private Limited-Mumbai, silymarin from Research-lab Fine Chem Industries, Mumbai. All chemicals and reagents used in this study were obtained from Sigma, St. Louis,, USA and Sisco Research Laboratories, Mumbai, India. Other chemicals which were used for analysis of various assay were obtained locally and were of analytical grade

Preparation of Ethanolic extract of *Capparis decidua*

Fresh fruits of *Capparis decidua* were obtained, identified and authenticated by Botanist and it is double washed with running water and then dried under shade. The dried fruit was thoroughly ground to a fine powder

using a blender. 100gms of ground powder was mixed with 500ml of ethanol which was then placed in shaker for 24hr and then 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 yielded around 20gms of ethanolic extract and the extract was then stored in a sterile container under -20°C.²⁸

Animals

Healthy female Wistar albino rats weighing around 180 \pm 20 g were used for the study. The animals were kept under standard temperature (21 \pm 2°C), under specific humidity, constant 12 h dark and 12 h light cycle as per National guidelines. The animals were maintained under sterilized paddy husk as bedding material in polypropylene cages and were fed with pellet and water *ad libitum*. And the study protocol received approval from IAEC (IAEC No. BRULAC/SDCH/SIMATS/IAEC/12-2019/037).

Experimental protocol

Thirty adult female albino rats of Wistar strain with 180 \pm 20 g body weight were randomly divided into five groups with six animals in each group. Group I animals serve as a control group and receive normal saline for a period of 14 days. Group II served as a methotrexate group which received methotrexate injection 20mg/kg body weight on the first day intraperitoneally followed by normal saline for a period of 14 days following IP methotrexate injection on the first day. Dose of methotrexate was fixed based on previous study.²⁹ Group III served as methotrexate with *Capparis decidua* 250mg/kg body weight group which received oral dose of ethanolic extract of *Capparis decidua* 250mg/kg body weight for a period of 14 days, Group IV served as methotrexate with *Capparis decidua* 500mg/kg body weight group which received oral dose of ethanolic extract of *Capparis decidua* 500mg/kg body weight for a period of 14 days. Ethanolic extract of *Capparis decidua* was dissolved in water for oral administration to the wistar rats. Group V served as methotrexate with Silymarin group which received oral dose of Silymarin 100mg/kg body weight in 0.5% CMC (positive control) for a period of 14 days following IP injection of methotrexate on the first day. Dose of *Capparis decidua* was decided based on the previous study.³⁰ Dose of silymarin (standard hepatoprotective agent) was decided based on previous literature.³¹ On the 15th day, the animals were anesthetized with sodium thiopentone (40 mg/kg body weight). The blood was collected by cardiac puncture. The serum obtained was

separated and stored under standard condition. Liver tissues were immediately dissected, weighed and stored at under standard temperature for analysis of various biochemical assays.

Biochemical assay

Liver function markers

Liver function test markers like ALT (Alkaline transaminase), AST (Aspartate Transaminase), ALP (Alkaline Phosphatase), were determined in serum using biochemical-assay, obtained from Spinreact, Spain. values were expressed in IU/L.

Lipid peroxidation

Lipid peroxidation (LPO) was measured in liver tissue homogenate as in accordance to the study by Devasagayam and Tarachand.³² The malondialdehyde (MDA) content was expressed as nmoles of MDA formed/min/mg protein. Hydrogen peroxide generation was done in accordance to the method of Pick and Keisari³³ and the values were expressed in μ moles/min/mg protein. For quantification of hydroxyl radical (OH^{*}), Puntarulo and Cederbaum,³⁴ method was used and values were expressed in the form of μ moles per min per mg protein.

Antioxidant enzymes

Rotruck *et al.*³⁵ method was used to assess GPx and the activity was expressed as μ g of utilized glutathione / per min per mg protein in liver tissue homogenate. The method of Habig *et al.*³⁶ was used to assess Glutathione-S-transferase (GST) activity and values were expressed in the form of μ moles of CDNB utilized per min per mg protein. The method of Staal *et al.*³⁷ was used to analyse Glutathione reductase (GR) and values for activity of GR and GSH were expressed in the form of nmoles of GSSG reduced per min per mg protein and nmoles of GSH per mg protein respectively.

mRNA expression analysis for Nrf2, HO-1, PPAR γ

Total RNA isolation

In the present study, we isolated total RNA by total RNA isolation reagent kit (TRIR, invitrogen). Briefly, 100 mg fresh tissue from control and treated animals were added to 1 ml of TRIR and homogenized. After, homogenization, 0.2 ml of chloroform was added and mixed the contents by vortexing for 1 min and kept for 5 min at 4°C. Then, it was centrifuged for 5 min at 12,000 \times g for 15 min at 4°C. Carefully, the upper aqueous phase was transferred carefully in to a fresh microfuge tube and isopropanol equal volume was added and placed on ice for 10 min after vortexing for 15 S. The supernatant was discarded after centrifugation

of the contents at 12000 \times g for 10 min at 4°C. After, the supernatant was discarded and by simple vortexing, the RNA pellet was washed with 1 ml of 75% ethanol. The total RNA concentration was measured spectrophotometrically and the concentration was expressed in micrograms.

cDNA conversion and real-time PCR analysis

Complementary DNA (cDNA) conversion was carried out from 2 μ g total RNA using reverse transcriptase kit, as per the manufacturer instructions (Eurogentec, Seraing, Belgium). For the analysis of real-time PCR, the PCR reaction mix contacting 2x reaction buffer (Takara SyBr green master mix), target gene specific and internal control gene and water and cDNA in total volume of 45 μ l and spun down (the primer sequences were listed in Table 1). The reaction was set up for 40 cycles (95°C for 5 min, 95°C for 5 s, 60°C for 20 s and 72°C for 40 s). The results were obtained and plotted by the PCR machine (CFX96 Touch Real-Time PCR Detection System) on a graph. Melt and amplification were used for quantification.

Protein expression analysis

Protein Isolation

In a buffer-A containing 100 mg tissues from control and treated animals were homogenized and centrifuged for 10 min at 4°C at 1300xg for 10 min at 4°C. After centrifugation, the supernatant was used as a total protein for the estimation of Nrf2, TNF- α , IL -1 β .

Western blot analysis

The proteins were separated on 10% gel by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Briefly, tissue lysate proteins (50 μ g/lane) were separated electrophoresed and transferred on polyvinylidenedifluoride (PVDF) membrane (Bio-Rad Laboratories Inc). The membranes were then blocked by 5% non-fat dry milk and then it was incubated in the

primary antibodies (1:1000 dilutions) for overnight. After, the membrane was washed with Tris-buffered saline – Tween-20 (TBS-T) and incubated for 1 h in horseradish peroxidase-conjugated secondary antibody (1:10000 dilution) and the specific signals were using enhanced chemiluminescence detection systems (Thermo Fisher scientific Inc, USA), the specific signals were detected using Chemidoc and quantified by Quantity One image analysis system (Bio-Rad Laboratories, CA). After, the protein bands were captured. As the invariant control in the present study, we used rat β -actin.

Statistical analysis

Data were expressed as mean \pm S.E.M and analyzed by one-way analysis of variance followed by Tukey's *post hoc* analysis multiple comparison test to determine the significance of differences between groups using Graphpad prism software. A *p*-value less than 0.05 was considered as showing significant difference.

RESULTS

Effects of *Capparis decidua* on liver function in hepatic tissue

As seen in Figure 1, liver function markers like ALT levels, AST levels and ALP levels were increased significantly in the methotrexate induced group of animals when compared to the control group of animals (*p* < 0.001). Oral administration of *Capparis decidua* at a dose of 250 mg/kg has significantly reduced methotrexate-induced

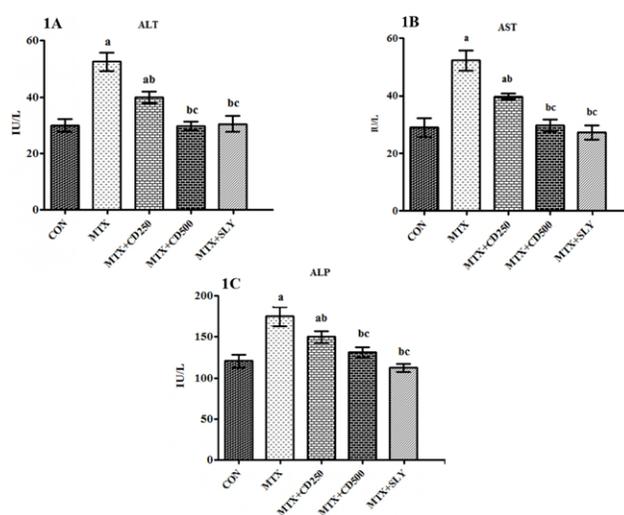


Figure 1(A, B, C): Effect of treatment with CD on AST, ALT and ALP levels in MTX-induced hepatotoxicity. Data are represented as means \pm SD. Data were analyzed by one-way ANOVA followed by Tukey's *post hoc* test for multiple comparisons. a - compared with the control, b compared with methotrexate induced group, c - compared with CD 250 treated group.

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

| Gene name | Forward primer (5'–3') | Reverse primer (5'–3') |
|---------------|---------------------------|---------------------------|
| Nrf2 | TTGTAGATGACCATGA GTCGC | TGTCCTGCTGTAT GCTGCTT |
| HO-1 | GTAATGCAGTGTTG GCCCC | ATGTGCCAGGCAT CTCCCTC |
| PPAR γ | GGACGCTGAAGAAGA GACCTG | CCGGTCTCTGTCT GAGTATG |
| GAPDH | TGGATTTGGACGCAT TGTC | TTTGCACTGGTACG TGTTGAT |

increase in AST levels, ALT levels and ALP levels ($p < 0.01$) (Figure 1 a, b and c). Oral administration of *Capparis decidua* extract at a dose of 500 mg/kg for a period of 14 days has reduced methotrexate-induced increase in all liver function parameters i.e AST levels, ALT levels and ALP levels ($p < 0.001$) significantly. The result was comparable with the silymarin.

Effects of *Capparis decidua* on lipid peroxidation

In group II rats, administration of IP injection of methotrexate produced a ($p < 0.001$) rise in the level of Lipid peroxidation, H₂O₂, OH radical when compared with control group of rats while group III rats, oral administration of with *Capparis decidua* 250mg/kg extract showed a ($p < 0.001$) reduction in the levels of LPO ($p < 0.01$), H₂O₂ ($p < 0.05$), OH radical ($p < 0.05$) in the hepatic tissue of rats significantly. Groups of rats namely IV and V, administered with *Capparis decidua* 500mg/kg and Silymarin, has caused a fall in the levels ($p < 0.001$) significantly compared with methotrexate administered group of rats (Figure 2 a, b, c)

Effects of *Capparis decidua* on antioxidants

Figure 3 (a, b and c) shows the effect of *Capparis decidua* on the antioxidants like GST, GPx, GR content in hepatic tissue. Administration of methotrexate has decreased GST, GPx and glutathione reductase content significantly when compared with the normal animals (all $p < 0.001$). Oral administration of *Capparis decidua*

250 mg/kg has increased these levels of GST levels ($p < 0.001$), GPx levels ($p < 0.01$), GR levels ($p < 0.01$) when compared with the methotrexate induced group of animals and it was statistically significant. Treatment with *Capparis decidua* 500 mg/kg has significantly increased the levels of Glutathione S Transferase, Glutathione peroxidase, glutathione reductase levels when compared

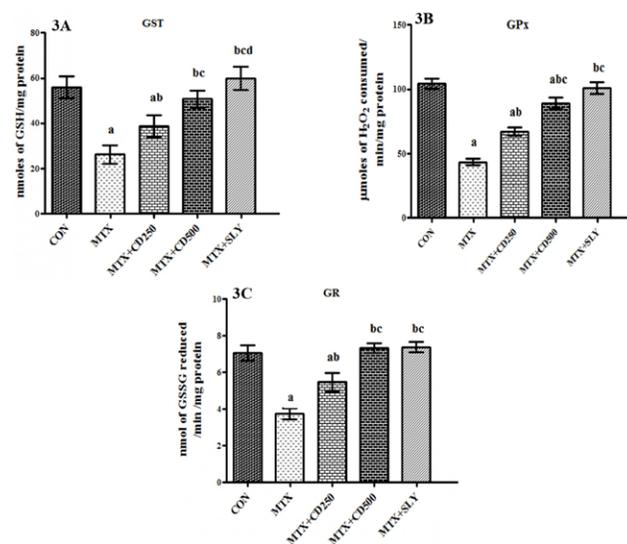


Figure 3 (A, B, C): Effect of CD on GST, GPx, GR in liver tissue of methotrexate intoxicated rats. Data are represented as means \pm SD. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. (mtx – methotrexate, CD – *Capparis decidua*). a - compared with the control, b compared with methotrexate induced group, c - compared with CD 250 treated group.

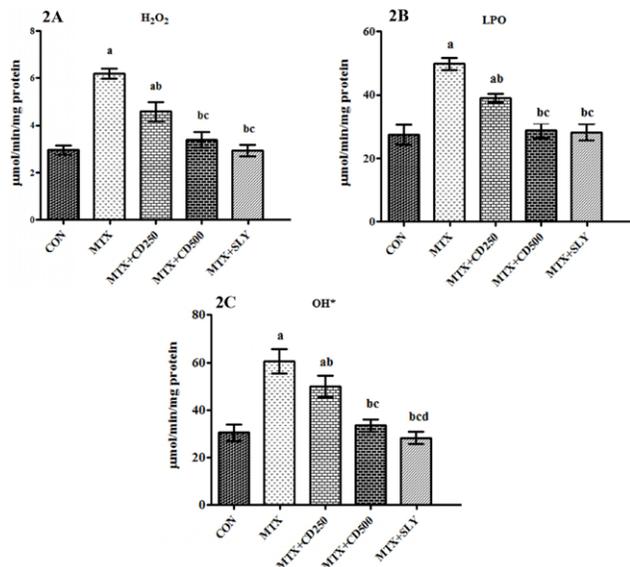


Figure 2(A, B, C): Effect of CD on H₂O₂, OH, LPO radical in liver tissue of methotrexate intoxicated rats. Data are represented as means \pm SD. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. (mtx – methotrexate, CD – *Capparis decidua*). a - compared with the control, b compared with methotrexate induced group, c - compared with CD 250 treated group.

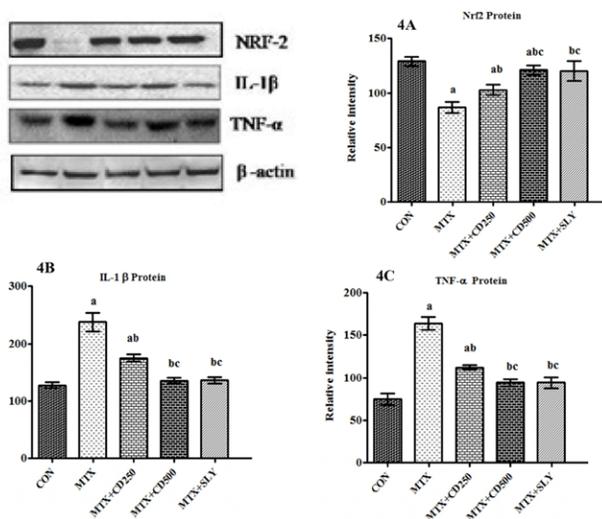


Figure 4 (A, B, C): Represents effect of CD on Nrf2, TNf alpha, IL -1 β in liver tissue of methotrexate intoxicated rats. Data are represented as means \pm SD. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. (mtx – methotrexate, CD – *Capparis decidua*). a - compared with the control, b compared with methotrexate induced group, c - compared with CD 250 treated group.

with the methotrexate administered group of animals ($p < 0.001$) (Figure 3 a-c).

Effect of *Capparis decidua* on mRNA expression of Nrf2, HO-1, PPAR γ

As shown in Figure 4 a, b, c the administration of methotrexate revealed significant decrease in the both mRNA of Nrf2, HO-1, PPAR γ in hepatic tissue when compared with the control group of animals ($p < 0.001$) and it was statistically significant, whereas *Capparis decidua* 250 and significantly caused up regulation of mRNA expression of Nrf2 levels ($p < 0.001$), HO-1 levels ($p < 0.001$) and it was highly significant statistically but there was decrease in PPAR γ but it was not statistically significant ($p > 0.05$) when compared with the methotrexate administered group *Capparis decidua* 500 significantly caused up regulation of mRNA expression of Nrf2, HO-1, PPAR γ , compared with the methotrexate administered group of rats ($p < 0.001$) (Figure 5 a-c).

Effects of *Capparis decidua* on Nrf2, TNF- α , IL -1 β

As shown in Figure 4 a, b, c the administration of methotrexate caused reduction in the levels of protein Nrf2 significantly and elevation in the TNF- α and IL -1 β levels in hepatic tissue when compared to the control group, whereas *Capparis decidua* 250 and 500 significantly caused significant increase in Nrf2 levels ($p < 0.001$) and caused down regulation of protein TNF- α and IL -1 β

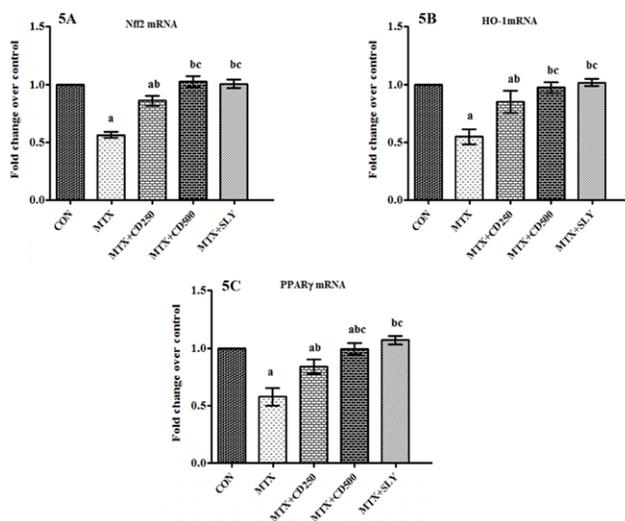


Figure 5: (A,B,C,D) Represents effect of CD on mRNA of Nrf2, HO, PPAR gamma in liver tissue of methotrexate intoxicated rats. Data are represented as means \pm SD. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. (mtx – methotrexate, CD – *Capparis decidua*). a - compared with the control, b compared with methotrexate induced group, c - compared with CD 250 treated group.

in hepatic tissue when compared with the methotrexate administered group of rats ($p < 0.001$) (Figure 4 a-c).

DISCUSSION

It is an important medicinal plant used to cure jaundice and other liver disorders in the traditional system of medicine.²⁷ The present study is an attempt to study its effect against methotrexate induced hepatotoxicity with special attention focusing on effect on Nrf2 signaling pathway. Methotrexate is a widely used anticancer immunosuppressant drug having its clinical application on diseases like acute lymphoblastic leukaemia, rheumatoid arthritis, ectopic pregnancy, crohn's disease, psoriasis, psoriatic arthritis, ulcerative colitis.^{38,39} Study shows that methotrexate increased levels of liver function markers like Alkaline transaminase, Aspartate Transaminase, Alkaline Phosphatase indicating liver damage with the previous study.⁴⁰ methotrexate induces liver damage through production of ROS leading to oxidative stress and also interferes with antioxidant defence mechanism.⁴¹ The present study showed that the methotrexate administration induced the lipid peroxidation which was shown by increase in parameters like H₂O₂, NO, TBARS levels in hepatic tissue. This was in accordance to the previous study which has also shown the elevated markers of lipid peroxidation.^{42,43} Our study showed that the administration of *Capparis decidua* fruit extract has effectively reduced hepatic levels of ALT, AST and ALP indicating its protective effect against liver damage induced by methotrexate. This was in accordance with the study²⁷ (Ali et al. 2009) which also showed that *Capparis decidua* stem extract reducing the liver function markers in CCl₄ induced hepatotoxicity. Administration of *Capparis decidua* ethanolic extract in methotrexate induced rats has reduced the elevated levels of lipid peroxidation markers like H₂O₂, NO, TBARS. The presence of antioxidants in *Capparis decidua*⁴⁴ could be responsible for attenuating lipid peroxidation induced by methotrexate. Previously it was studied that *Capparis decidua* fruit extract was found to be effective against alloxan induced lipid peroxidation (LPO) in erythrocyte, tissues of heart and kidney.⁴⁵ Our present study also showed that the methotrexate administration caused significant reduction in GSH, GPX, GR, levels in hepatic tissue which was in accordance with the previous findings.⁴⁶ It has been studied that methotrexate decreases the NADPH availability by inhibiting cytosolic nicotinamide adenosine diphosphate (NADP) dependent dehydrogenase and NADP malic enzyme,⁴⁷ NADPH is required to maintain the important cytosolic antioxidant glutathione, under

reduced state, by the enzyme glutathione reductase. Methotrexate promotes significant reduction in glutathione levels (GSH) thereby sensitizing the cells to the action of reactive oxygen species.^{48,49} Glutathione peroxidase protects tissues from oxidative stress damage by converting hydrogen peroxide in the presence of GSH to water and disulfide glutathione (GSSG). GSSG formed as a result, will be converted back to GSH with the help of glutathione reductase. The present study also reported that administration of *Capparis decidua* fruit extract has replenished the levels of GSH, GPx, GR. This was comparable to the previous study by an author who showed that administration of *Capparis decidua* has promoted increase in levels of Gpx, GSH, GR reduced glutathione in renal and heart tissue of alloxan induced diabetic rats.⁵⁰ However previously, there are no studies of *Capparis decidua* in methotrexate induced hepatotoxicity.

Previous studies have reported that methotrexate induced liver injury is attributed to inflammation.^{42,43} Our findings also revealed increased serum levels of protein tumour necrosis factor α and Interleukin 1 β levels in the methotrexate administered rats. This could be due to activation of NF κ B signalling pathway⁵¹ which promotes activation of inflammatory markers. *Capparis decidua* prevented methotrexate induced inflammatory response which was shown by decrease in TNF α and IL-1 β levels. This could be due to suppression of NF κ B pathway by the presence of alkaloids, flavanoids, terpenes of the extract which suppresses the inflammatory proteins. The studies have shown that chloroform and methanolic extract of aerial parts of *Capparis decidua* possess potent anti-inflammatory activity against induced paw oedema in Swiss albino rats.⁵²

To understand the mechanism behind the hepatoprotective effect of *Capparis decidua* against methotrexate, Nrf2/HO-1 and PPAR γ expression were studied. Nrf2 will be in a deactivated state in cytosol and it get separated from Keap-1, translocates to nucleus, activates the transcription of antioxidant gene by combining to ARE under situation of oxidative stress.⁵² PPAR γ is reported to protect against drug induced liver toxicity by suppressing oxidative stress, inflammation and apoptosis.^{53,54} Our findings showed that the methotrexate administration down regulated the mRNA expression of Nrf2 and Nrf2 protein. It was confirmed by reduced expression of HO-1 mRNA.²² Methotrexate administration also significantly downregulated mRNA expression of PPAR γ . *Capparis decidua* administration has upregulated the levels of Nrf2 mRNA, Nrf2 protein HO-1 mRNA expression and PPAR γ mRNA. This could be attributed to the presence of phytoconstituents of

the extract which could be responsible for upregulation of Nrf2 and PPAR γ pathway. No studies have focused the mechanism of hepatoprotective effects of *Capparis decidua* through Nrf2 and PPAR γ pathway.

The present study has demonstrated that *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 terpenoids, alkaloids, glycosides, flavonoids present in the extract which act as natural antioxidants and that could be responsible for increasing defense mechanisms against free radical production induced by methotrexate administration.

CONCLUSION

Thus, the extract has shown the promising biological activity against methotrexate induced hepatotoxicity. *Capparis decidua* fruit extract protected against hepatic damage, inflammation induced by methotrexate through up-regulation of Nrf2/HO-1 and PPAR γ signaling pathways. Hepatoprotective effect of the *Capparis decidua* fruit is therefore attributed to its antioxidant effects.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MTX: Methotrexate; **ROS:** Reactive oxygen species; **Nrf2:** Nuclear factor erythroid derived – 2; **HO:** 1 – Hemeoxygenase; **PPAR γ :** Peroxisome proliferator activated receptor gamma; **NF- κ B:** Nuclear factor-kappa B; **TNF- α :** Tumour necrosis factor alpha IL-1 β - interleukin-1beta; **C. Decidua:** *Capparis decidua*; **ALT:** Alkaline transaminase; **AST:** Aspartate Transaminase; **ALP:** Alkaline Phosphatase; **MDA:** Malondialdehyde; **GST:** Glutathione S Transferase; **GPx:** Glutathione peroxidase; **GR:** Glutathione reductase.

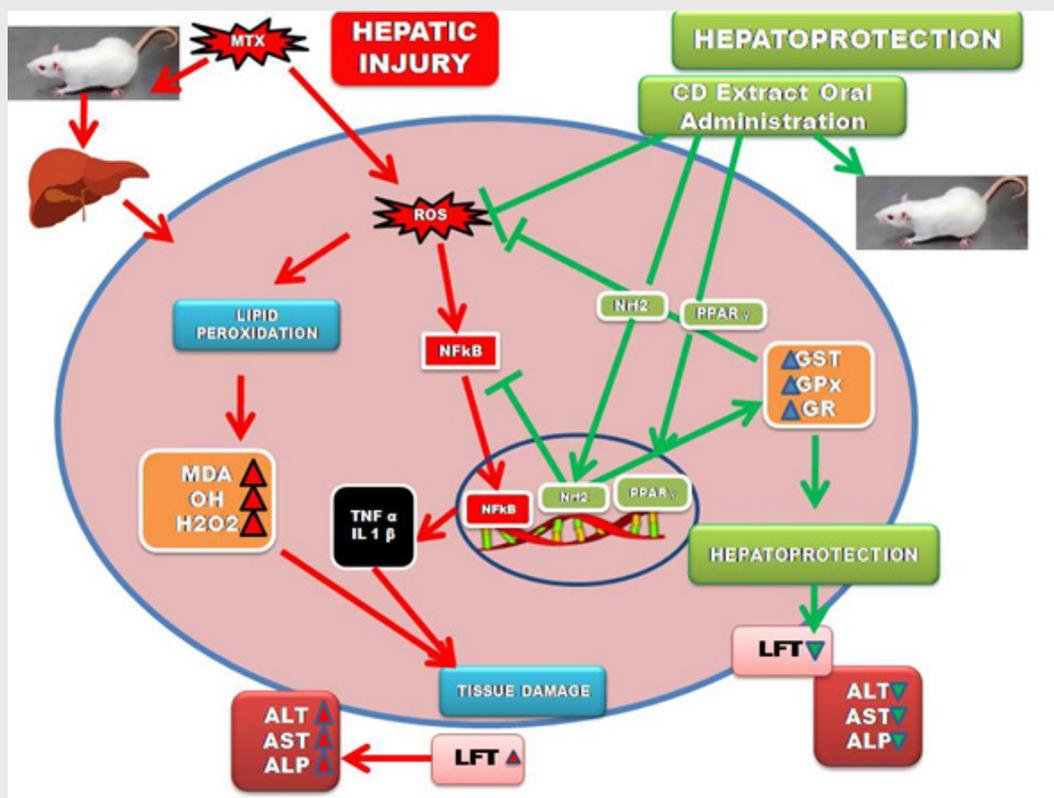
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PICTORIAL ABSTRACT



A proposed schematic diagram illustrating the protective mechanism of *Capparis decidua* against methotrexate-induced Hepatotoxicity via Nrf2/HO-1 and PPAR γ mediated pathway
 MTX - methotrexate, ROS - reactive oxygen species, Nrf2 - Nuclear factor erythroid derived - 2, HO- 1 - Hemeoxygenase, PPAR γ - Peroxisome proliferator activated receptor gamma, NF- κ B - nuclear factor-kappa B, TNF α - Tumour necrosis factor alpha IL-1 β - interleukin-1beta, -CD- *Capparis decidua*, ALT - Alkaline transaminase, AST - Aspartate Transaminase, ALP - Alkaline Phosphatase, MDA - Malondialdehyde, GST - Glutathione S Transferase, GPx - Glutathione peroxidase, GR - Glutathione reductase.

SUMMARY

- *Capparis decidua*, a very important medicinal plant of India which is used in traditional system of medicine.
- The study shows that the ethanolic extract of *Capparis deciduas* fruit 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.
- Hepatoprotective property of the fruit could be via activation of Nrf2,/HO-1 and PPAR γ signaling pathways.

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