**INTRODUCTION**

The liver is a vital organ that helps in the conjugation and detoxification of a variety of pharmaceuticals. On the other hand, xenobiotics and pathogens frequently disrupt its operation. Cirrhosis or malignant lesions can arise from persistent or severe xenobiotic exposure if left untreated.1,2 Millions of people worldwide are affected by hepatic damage caused by alcohol, drugs, and infections. As a result, acute and chronic liver diseases continue to be significant global health concerns3,4. Paracetamol, CCl4, nitrosamines, and polycyclic aromatic hydrocarbons are all known to induce significant liver damage. Additional hepatoprotective medications or safer alternatives to currently available drugs are urgently needed.5 Modern medicine’s alternatives are limited due to the unreliability and inefficiency of existing options. According to the literature, liver-toxic chemicals, ionising radiations, environmental pollutants, and drug exposure all produce substantial amounts of oxygen free radicals such as superoxide anion radical (O2•−) which cause hepatotoxicity.6,7 CCl4 is a common solvent in the chemical industry. It’s a well-studied xenobioc-induced free radical-mediated hepatotoxicity model in animals. CCl4 damages the liver through various methods.8 Increased lipid peroxidation due to increased free radical generation produced by CCl4 is one of the processes that contribute to hepatotoxicity. Infiltrating inflammatory cells to the injury site8 activates immune systems as well. Immune cells may thus be responsible for the secretion of pro-inflammatory cytokines such as TNF-α and IL-6, which enhance hepatotoxicity by prolonging the inflammatory cycle.9

α-Asarone is a phenylpropane biosynthesized in a variety of herbs, spices, and medicinal plants via the shikimate pathway10, Asarone is a plant in the Aristolochiaceae (Asarum europaeum Linne) and Acoraceae families that belong to the aristolochiaceae (Asarum europaeum Linne) and Acoraceae families (Acorus). α-Asarone is a bioactive natural chemical discovered in the rhizome of Acorus tatarinowii11, α-Asarone is a drug used to treat hyperlipidemia. Acorus calamus Linn extracts α-Asarone and their isomers have been shown to have antidepressants, antihyperlipidemic, anti-cholestatic, anti-inflammatory, anticancer, anticonvulsive, antibacterial, and antiviral effects.2,12,13 The objective of the study was to the protective effect of α-asarone in CCL4-induced hepatotoxicity in rats.

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**Abstract**

The present study aimed to study the protective effect of alpha-Asarone in CCL4-induced hepatotoxicity in experimental rats. The rats were randomly divided into 5 groups each containing six rats. CCL4 was given at a dosage of 0.5 mL/kg to produce hepatotoxicity, and serum AST, ALT, total bilirubin, albumin, as well as hepatic hydroxyproline (HP), reduced glutathione (GSH) and malondialdehyde (MDA), cytokines, and NO, were assessed. CCL4 treatment resulted in a decrease in body weight and an increase in liver weight in rats, while treatment with α-Asarone resulted in normal body and liver weight. Serum AST, ALT, total bilirubin, HP, GSH, MDA, and cytokines were increased in CCL4 treated rats. α-Asarone-treated rats showed a reduction in oxidative stress as well as inhibited the release of cytokines in dose dependent manner and showed protection against hepatotoxicity. From the study, we conclude that α-Asarone has a protective effect against the hepatotoxicity induced by CCL4.

**Keywords:** CCL4; Hepatotoxicity; ALT; oxidative stress; α-Asarone; Cytokine.

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**A-Asarone Protects CCL4 Induced Hepatotoxicity in Experimental Rats by inhibiting oxidative stress and cytokines**

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**Original Research Article**

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MATERIAL AND METHODS

Animals

For this investigation, albino Wistar rats weighing 180-200 grams were used. Animals were housed in metabolic cages with free access to normal rat feed and water under standard conditions of room temperature (22-240°C) and relative humidity (65%) with a 12 hour light/dark cycle. IAEC officially approved the experimental protocol. The protocol approval no is 751/PO/Re/S/03/CPCSEA

Drugs and chemicals

Alpha Asarone was purchased from Sigma USA. ELISA kits for TNF-α, IL-6, and IL-1β, were purchased from eBioscience, USA., AST, ALP, and ALT kits were procured from Erba diagnostics, India. All other reagents used were of analytical grade.

Experimental design

Animals were randomly divided into five groups each containing six rats.

Group 1: Normal - Each animal receives 1ml vehicle
Group 2: Control - Received CCL4 (0.5 mL/kg)
Group 3: A100- α-Asarone (100 mg/kg/day)
Group 4: A150- α-Asarone (150 mg/kg/day)
Group 5: A200- α-Asarone (200 mg/kg/day)

Hepatotoxicity was induced in all rats except the normal group by injecting a single dose 0.5 mL/kg of CCL4 on the first day of the study.

Collection of blood samples

A mild ether anaesthetic was used to anaesthetize the rats. After each animal’s blood was taken by the retro-orbital method in Eppendorf tubes, the serum was separated by spinning at 10000 rpm for 10 minutes in a chilled centrifuge. The serum samples were stored at -20°C until analysis

Experimental parameters

On the first and seventh days, the body weight of each group rat was measured with a weighing balance, and the liver weight of each rat was tested at the end of the experiment after scarification with a high dose of ether anaesthesia. The livers of each rat were removed and rinsed in ice-cold phosphate-buffered saline before being weighed and blotted on filter paper. For the estimation of biochemical parameters, a 10% liver tissue homogenate was produced.

Biochemical parameters

Serum biochemical parameters like AST, ALP, ALT, TB, and HP were determined as the procedure mentioned in the literature and manufacturer’s instruction manual

Estimation of oxidative Stress

Antioxidant parameters like SOD, GSH, Catalase, and MDA were estimated by procedures described in published literature

Determination of Nitric oxide

Griess reagent (% sulfanilamide, 0.1 percent naphthyl ethylenediamine dihydrochloride, 2.5 percent H3PO4) and homogenate supernatant (500 L), followed by a 10-minute incubation at room temperature. The absorbance was measured at 540 nm. The amount of nitrite, which was represented as M/mg of protein, was calculated using a sodium nitrite (NaNO2) standard curve

Determination of cytokine level

According to the manufacturer’s protocol, ELISA kits were used to measure the amounts of cytokines such as IL-6, IL-1, and TNF-α in liver tissue homogenate. The final concentration was calculated using a standard curve

RESULTS

Effect of α- Asarone on body weight and liver weight

When compared to normal groups, the disease control group’s body weight decreased during the treatment period. The group given α-Asarone (200 mg/kg) have the same pattern of weight gain as the control group. In comparison to the control group, α-Asarone at 100 mg/kg does not affect body weight. At the end of the investigation, the liver weight of the animals was measured, and we found that the control group had a higher liver weight than the normal group. α-Asarone at 200 mg/kg had the largest effect on liver weight when compared to the control group.

Table 1: Effect of α- Asarone on body weight and liver weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Bodyweight (gm)</th>
<th>Liver weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Day</td>
<td>7 day</td>
</tr>
<tr>
<td>Normal</td>
<td>189.8± 9.62</td>
<td>194.8±10.54</td>
</tr>
<tr>
<td>CCL3</td>
<td>181.6± 7.59</td>
<td>168.6±11.12***</td>
</tr>
<tr>
<td>A100</td>
<td>186.6±8.46</td>
<td>189.8±8.57**</td>
</tr>
<tr>
<td>A150</td>
<td>187.9±8.27</td>
<td>191.2±6.71***</td>
</tr>
<tr>
<td>A200</td>
<td>186.8±7.19</td>
<td>192.5±6.84***</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SEM, analyzed using one-way analysis of variance, *p<0.05, **P<0.01, ***P<0.001 compared to control rats, and #P<0.01, ###P<0.001 is compared with the sham animals.
**Effect of α-Asarone on biochemical parameters**

In comparison to normal rats, the present study found a substantial increase in serum AST, ALP, ALT, total bilirubin, and HP in the control group. Figures 1 and 2 show that rats were given a 100 mg/kg dose of α-Asarone did not affect biochemical marker levels, whereas rats given 150 and 200 mg/kg α-Asarone had a decrease in biochemical marker levels when compared to the control group. The increase in biochemical levels in the control group rats indicated that the rats' livers were toxic.

![Figure 1: Effect of α-Asarone on biochemical parameters](image1)

Data were expressed as means ± SEM, n = 06. Statistical significance was determined by one-way ANOVA followed by the Dunnet test: Compared with Normal ***P < 0.01, Compared with Control

![Figure 2: Effect of α-Asarone on biochemical parameters](image2)

Data were expressed as means ± SEM, n = 06. Statistical significance was determined by one-way ANOVA followed by the Dunnet test: Compared with Normal ***P < 0.01, Compared with Control

**Effect of α-Asarone on serum cytokine**

The effect of α-Asarone on pro-inflammatory cytokine production of IL-6, IL-1, and TNF- in the serum was studied. When compared to the normal group, the control group had higher levels of pro-inflammatory cytokines such as IL-6, IL-1, and TNF- at the end of the study. Treatment with α-Asarone (200 mg/kg) for 7 days reduced the levels of IL-6, IL-1, and TNF- to near-normal levels, showing that α-Asarone inhibits cytokine release. In the case of IL-10, the level was higher in the control group than in the normal group. In comparison to the control group illustrated in figure 3, rats administered with α-Asarone had a dose-dependent effect.

![Figure 3: Effect of α-Asarone on cytokine level](image3)

Data were expressed as means ± SEM, n = 06. Statistical significance was determined by one-way ANOVA followed by the Dunnet test: Compared with Normal ***P < 0.01, Compared with normal; *P<0.05; ***P<0.001 compared to Control
Effect of α-Asarone on oxidative stress

CCL4 induced liver fibrosis rats showed a significant decrease in the level of SOD and GSH when compared to the normal group. Treatment with α-Asarone (100, 150, and 200 mg/kg) for 7 days showed dose-dependently increased the level of SOD and GSH when compared with CCL4 treated group. CCL4 induced liver toxicity revealed a decrease in the level of GSH when compared to the Normal group. Treatment with α-Asarone (100, 150, and 200 mg/kg) for 7 days shows a dose-dependently decreased the level of MDA when compared with the disease control group. α-Asarone treated at a dose 200 mg/kg showed a most prominent effect on MDA level as compared to the control group. CCL4 induced liver toxicity revealed a decrease in catalase activity when compared to the Normal group. Oral treatment with α-Asarone (150 and 200 mg/kg) for 7 days shows a dose-dependently significantly increased the catalase activity when compared with CCL4 treated group shown in figure 4.

![Figure 4: Effect of α-Asarone on oxidative stress](image)

Data were expressed as means ± SEM, n = 06. A: Lipid Peroxidation; B: GSH; C: Catalase; D: SOD. Statistical significance was determined by one-way ANOVA followed by the Dunnet test: Compared with Normal ###P < 0.01, Compared with Control

Effect of α-Asarone on nitric oxide level

When we were treated with CCL4 shows a significant increase in the level of nitric oxide as compared to the normal group. Rats treated with α-Asarone at different dose shows a decrease in the level of nitric oxide dose-dependently. Rats treated with α-Asarone 200 mg/kg shows a significant decrease in the level of nitric oxide as compared to CCL4 treated rats shown in figure 5.

![Figure 5: Effect of α-Asarone on nitric oxide](image)

DISCUSSION

The present study aimed to investigate the protective effect of α-Asarone in CCL4 induced hepatotoxicity in experimental animals. The ideal model is CCl4-induced liver injury, which causes hepatic alterations similar to cirrhosis/hepatitis, such as mononuclear cell infiltration and steatotic foamy degeneration of hepatocytes. Carbon tetrachloride-induced toxicity is characterised by the generation of reactive intermediate trichloromethyl radical and trichloromethyl peroxy radicals, which alkylate cellular proteins and other macromolecules while simultaneously targeting polyunsaturated fatty acids. They are thought to form lipid peroxides that cause hepatotoxicity, such as conjugated dienes, lipid hydroperoxides, malonaldehyde-like molecules, and other short-chain hydrocarbons.

In this study, we found that the bodyweight of CCL4 treated rats shows a reduction in body weight, and meanwhile treatment with α-Asarone increases the body weight of the rats. In the case of liver weight, it was increased in the CCL4 treated rats and maintained in the α-Asarone treated rats. The study showed that at the end of the study level of serum ALT, AST, TB, and TP were increased. However, the rats treated with α-Asarone were shows the normal level of all these as compared the normal.
A considerable imbalance between the creation and clearance of reactive oxygen species is classified as oxidative stress (ROS). This could be due to an overabundance of these chemicals or a decrease in antioxidant defences caused by CCL4 poisoning15. CCL4-treated rats exhibit oxidative damage, whereas α-Asarone-treated rats exhibit the opposite impact on oxidative damage. Hepatotoxicity is characterized by an imbalance of nitric oxide levels. In this study, CCL4-treated rats have higher levels of nitric oxide than rats treated with α-Asarone. The amount of cytokine in rats treated with CCL4 increased, whereas the level of cytokine in Asarone-treated rats decreased. According to the findings, Asarone has a protective effect against CCL4-induced hepatotoxicity in rats by regulating biochemical indicators and maintaining antioxidant enzyme and cytokine levels. We will undertake experiments on cell lines in the future to investigate the mechanism of α-Asarone in hepatotoxicity protection.

CONCLUSION

Alpha asarone is having a protective role in the chemical-induced hepatotoxicity in rats. It reduces oxidative and nitrosative stress as well as reduced the concentration of release of inflammatory cytokines.

Conflict of interests

All authors have no conflicts of interest to declare.

REFERENCES

22. Alam MF, et al., Therapeutic potential of Vanillylacetone against CCl4 induced hepatotoxicity by suppressing the serum marker, oxidative stress, inflammatory cytokines and apoptosis in Swiss albino mice. Experimental and molecular pathology, 2018; 105(1):81-88. https://doi.org/10.1016/j.expmed.2018.06.001