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Original Article

Alpha-Asarone Ameliorates Cisplatin Induced Hepatotoxicity *via* Reducing the Oxidative Stress in Experimental Animals

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ABSTRACT

Background: The aim of present study was to find protective effect of α-asarone in cisplatin-induced hepatotoxicity in experimental rats. **Materials and Methods:** The rats were divided into 5 groups at randomly. Cisplatin was given at a dosage of 7.5 mg/kg to produce hepatotoxicity, and serum AST, ALT, total bilirubin, and albumin, as well as hepatic hydroxy proline (HP), reduced glutathione (GSH), and malondialdehyde (MDA), cytokines, and NO, were assessed. Finally, histological examination of liver tissue was performed (H&E staining). **Results:** Cisplatin treatment resulted in a reduction in body weight and a rise in liver weight in rats, while treatment with -asarone resulted in normal body and liver weight. Serum AST, ALT, total bilirubin, HP, GSH, MDA and cytokines were increased cisplatin rats. α-Asarone treated rats showed reduction in the oxidative stress as well as inhibited the release of cytokines in dose dependent manner and showed protection against hepatotoxicity. Cisplatin treated rat's shows pathological tissue structure, while rats treated with α- asarone shows normal structure of liver tissue. **Conclusion:** The study concludes that, α-asarone has protective effect against the hepatotoxicity induced by Cisplatin.

Key words: Cisplatin, Hepatotoxicty, ALT, Oxidative stress, α-Asarone, Cytokine.

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INTRODUCTION

Cisplatin is an antineoplastic medication that is used to treat metastatic cancer and other solid tumours. Although larger cisplatin doses are more efficient in suppressing cancer, they produce permanent liver damage and other organ-adverse effects.[1] It's possible that the medication accumulates in hepatic tissue in excessive amounts, particularly when administered in large dosages. Cisplatin's clinical usage is generally restricted by its significant toxic side effects, which interfere with its therapeutic efficacy.[2,3] Cisplatin harmed the liver, most likely due to increased expression of CYP2E1, a potent reactive oxygen species generator. Hepatotoxicity has been linked to low doses of cisplatin administered frequently, most likely due to liver accumulation.[4,5] Nitrosative stress in hepatotoxicity is caused by reactive oxygen and nitrogen species (RNS) such as ONOO-, peroxinitrous acid (HONOO-), nitrogen dioxide radical (NO_2*), and other species. NO* is generated by a series of NOS enzymes that catalyse the production of NO*.[6] RNS is formed from NO*. -asarone is a phenylpropene biosynthesized via the shikimate pathway in a range of herbs, spices, and medicinal plants.[7] α-Asarone is a plant that belongs to the Aristolochiaceae (*Asarum europaeum* Linne') and Acofaceae families (Acorus). α-Asarone is a potent bioactive natural substance found in *Acorus tatarinowii's* rhizome.[8] α-Asarone is a hyperlipidemia treatment medication. The antidepressant, anti-hyperlipidemic, anticholestatic, anti-inflammatory, anticancer, anticonvulsive, antibacterial, and antiviral properties of *Acorus calamus* Linn extracts α-asarone and their isomers have been reported.[9,10] The aim of the research was to see whether asarone could protect rats against cisplatin-induced hepatotoxicity.

MATERIALS AND METHODS

Animals

Swiss Albino rats weighing between 180-250 gm were selected for this study. Animals were housed in metabolic cages under standard conditions of room temperature (22-24°C) and relative humidity 65% with12 hr light/dark cycle with free access to standard rat feed and water. The experimental protocol approved by IAEC. The protocol approval no is 751/PO/Re/S/03/CPCSEA.

Drugs and Chemicals

Cisplatin obtained from local pharmacy vender, 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 was of analytical grade.

Induction of Hepatotoxicity in Rats

Hepatotoxicity was induced in rats by injecting single dose 7.5 mg/kg of Cisplatin on 2nd day of study (Cisplatin diluted up to 1 ml by using distilled water).[11]

Experimental Design

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

Group 1: Normal - Each animal receives 1ml vehicle

Group 2: Control - Received cisplatin (7.5 mg/kg.) on 2nd day of study

Group 3: A100- α-Asarone (100 mg/kg/day) + cisplatin (7.5 mg/kg.) on 2nd day of study

Group 4: A150- α-Asarone (150 mg/kg/day) + cisplatin (7.5 mg/kg.) on 2nd day of study

Group 5: A200- α-Asarone (200 mg/kg/day) + cisplatin (7.5 mg/kg.) on 2nd day of study

Collection of Blood Samples

Light ether anaesthesia was used to anaesthetize the rats. Each animal's blood was taken via the retro-orbital route in eppendorf tubes and separated serum by centrifugation at 10000 rpm for 10 min using a cooling centrifuge. Serum samples were stored at -20°C until further analysis.

Experimental Parameters

The body weight of each group rat was measured using a weighing balance on the first and tenth days, and the liver weight of each rat was assessed at the end of the experiment following scarification with a high dosage of ether anaesthesia. Each rat's liver was removed and rinsed in ice-cold phosphate buffered saline before being blotted on filter paper and weighed. One part of the liver tissue was used for histopathology, while the other was used to prepare the homogenate.

Biochemical Parameters

Serum biochemical parameters like AST, ALP, ALT, TB and HP was determined as the procedure mentioned in the manufacturer's instruction manual.[12]

Estimation of Oxidative Stress

Estimation of Malondialdehyde of Lipid Peroxidation in Liver Tissue

The concentration of malondialdehyde (MDA) in liver tissues is determined using the previously published method, which is the most important indicator of membrane lipid peroxidation. The production of pink colour owing to the reaction between MDA and thiobarbituric acid was the basis of lipid peroxidation. At 532 nm, the absorbance of pink colour was determined spectrophotometrically.[13,14]

Estimation of Reduced Glutathione (GSH)

Glutathione concentration in liver tissues homogenate was determined as previously described method by Jain *et al.*, 2020.[15]

Estimation of Superoxide Dismutase (SOD) Activity

The liver homogenate (10 µl) was added in mixture of 20 µl of 500 mM/1 of sodium carbonate, 1 ml of 0.3% Triton X-100, 10 µL of 1.0 mM/1 of EDTA, 2.5 ml of 10 mM/1 of hydroxylamine, and 89 ml of distilled water. To this reaction mixture, 10 µl of 240 µM/1 of NBT was added and finally optical density of this reaction mixture was measured at 560 nm in kinetic mode.[16]

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

Group	Body weight (gm)		Liver weight (gm)
	0 Day	28 day	
Normal	185.2± 8.53	197.5±9.46	4.65 ± 0.7
Control	184.9± 4.9	248.9±13.18	7.03 ± 0.8$^{###}$
C100	187.5±6.94	220.4±11.57	6.49 ± 1.3
C300	186.4±7.18	218.5±9.43	6.03 ± 1.1**
C500	182.7±10.81	216.4±10.27	5.32 ± 0.9***

Data were expressed as mean ± SEM, analysed 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.

Determination of Nitric Oxide

Supernatant from homogenate (500 µL) and Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride, 2.5% H_3PO_4) followed by incubation at room temperature for 10 min. The absorbance was measured at 540 nm. The amount of nitrite was calculated from a sodium nitrite (NaNO2) standard curve and was expressed as µM/mg of protein.[1]

Determination of Cytokine Level

The concentrations of cytokine like IL-6, IL-1β and TNF-α in liver tissue homogenate was determined according to the manufacturer's protocol using ELISA kits. Final concentration was determined by using standard curve.[17]

Histopathology

At the end of study, animals were anesthetized by using urethane and liver was dissected from each rat and stored in 10 % formalin solution and prepared paraffin blocks. Thin sections were taken from every block and stained with hematoxylin and eosin and finally observed under microscope for liver fibrosis and photographs were taken by using motic camera system.[18]

RESULTS

Effect of α- asarone on Body Weight and Liver Weight

Body weight of disease control group increased throughout the treatment periods as compared to normal groups. In group treated with α-asarone (200 mg/kg) shows same pattern of increase in body weight like normal group. α-Asarone treated with 100 mg/kg has no effect on body weight as compared to control group (P< 0.005). Liver weight of animal was measured at the end of study and we found that increase in liver weight in control group as compared to normal group (P<0.005). α-Asarone treated with 500 mg/kg showed most prominent effect on liver weight as compared to control group (P< 0.005) shown in Table 1.

Effect of α-asarone on Biochemical Parameters

The present study showed that significant increase in the level of serum AST, ALP, ALT, total bilirubin and HP in control group as compared to normal rats (P < 0.001). Rat treated with 100 mg/kg dose of α-asarone has no effect on biochemical markers level but rat treated with 150 and 200 mg/kg α-asarone showed decrease in level of biochemical markers as compared to the control group (P< 0.005) shown in Figure 1 and 2. Increase in biochemical level in control group rats indicated the liver toxicity in rats. In group treated with only α-asarone 200 mg/kg showed no effect on level of biochemical markers.

Effect of α-asarone on Serum Cytokine

The effect of α-asarone was determined on rise in pro- inflammatory cytokine expression of IL-6, IL-1β and TNF-α in the serum. It was found that increased level of pro-inflammatory cytokines of IL-6, IL-1β and TNF-α in control group at the end of study as compared to normal group. Treatment with α-asarone (200 mg/kg) for 10th days significantly decreased the level of IL-6, IL-1β and TNF-α levels almost to the normal levels indicating that α-asarone exerts an inhibitory effect on the cytokine release. In case of IL-10, the level increased in control group as compared to normal group. Rats treated with α-asarone shows dose dependent effect as compared to control group shown in Figure 3.

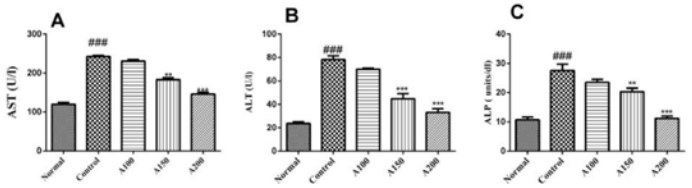

Figure 1: Effect of α- Asarone on biochemical parameters.
Data were expressed as means ± SEM, *n* = 06. Statistical significance was determined by one-way ANOVA followed by the Dunnet test: Compared with Normal $^{###}P < 0.01$, Compared with Control

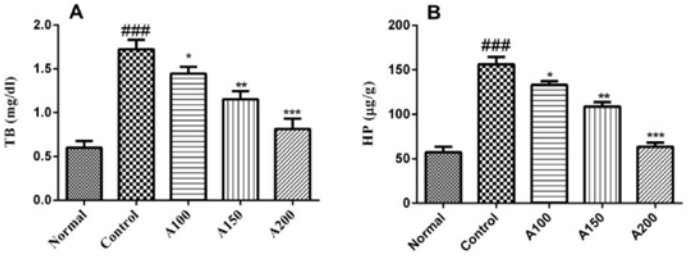

Figure 2: Effect of α- Asarone on biochemical parameters.
Data were expressed as means ± SEM, *n* = 06. Statistical significance was determined by one-way ANOVA followed by the Dunnet test: Compared with Normal $^{###}P < 0.01$, Compared with Control.

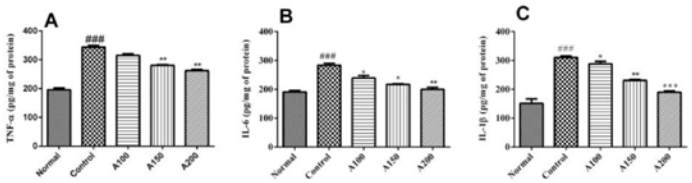

Figure 3: Effect of α- Asarone on cytokine level.
Data were expressed as means ± SEM, *n* = 06. Statistical significance was determined by one-way ANOVA followed by the Dunnet test: Compared with Normal $^{###}P < 0.01$, Compared with normal; $^{*}P<0.05$; $^{***}P<0.001$ id compared to Control

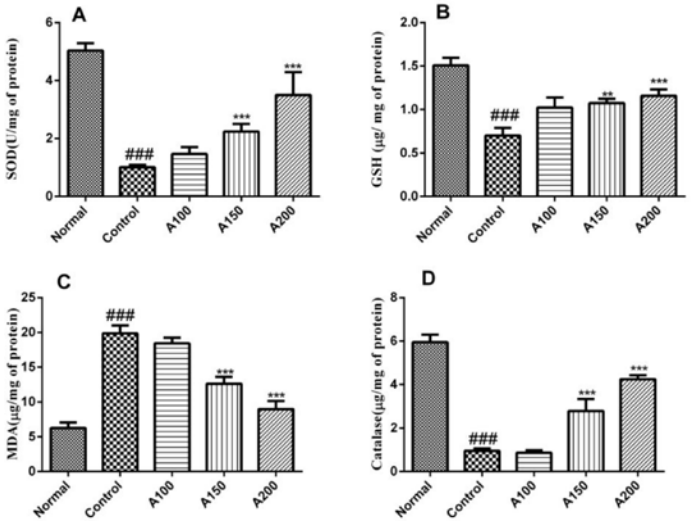

Figure 4: Effect of α- Asarone on oxidative stress.
Data were expressed as means ± SEM, *n* = 06. A: Lipid Peroxidation; B: GSH; C: Catalase; D: SOD. Statistical significance was determined by one-way ANOVA followed by the Dunnet test: Compared with Normal $^{###}P < 0.01$, Compared with Control

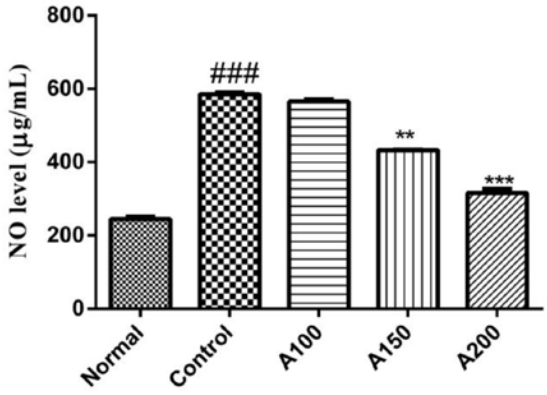

Figure 5: Effect of α- Asarone on nitric oxide.

Effect of L-Carnitine on Oxidative Stress

Cisplatin induced liver fibrosis rats showed a significant decrease in level of SOD and GSH, when compared to normal group. Treatment with α-asarone (100, 150 and 200 mg/kg) for 10 day showed dose dependently increased the level of SOD and GSH when compared with cisplatin treated group. Cisplatin induced liver toxicity revealed a decrease in level of GSH when compared to Normal group. Oral treatment with α-asarone (100, 150 and 200 mg/kg) for 10 days shows dose dependently decreased the level of MDA when compared with disease control group. α-Asarone treated at dose 200 mg/kg showed most prominent effect on MDA level as compared to control group. Cisplatin induced liver toxicity revealed a decrease in catalase activity when compared to Normal group. Oral treatment with α-asarone (150 and 200 mg/kg) for 10 days shows dose dependently significantly increased the catalase activity when compared with Cisplatin treated group shown in Figure 4.

Effect of α-asarone on Nitric Oxide Level

When we treated with Cisplatin shows significant increase in the level of nitric oxide as compared to normal group. Rats treated with α-asarone with different dose shows decrease in the level of nitric oxide dose dependently. Rats treated with α-asarone 200 mg/kg shows significant decrease in the level of nitric oxide as compared to Cisplatin treated rats shown in Figure 5.

Effect of α-asarone on Histopathology of Liver

The normal and sham groups liver tissues shown to have normal structure and architecture. The animals with BDL showed marked damage to hepatocytes as observed with edema, neutrophil infiltration and vacuoles. Treatment with α-asarone at the entire dose range showed less edema and fibrosis as observed in the Figure 6.

DISCUSSION

Present study aimed to investigation of protective effect of α-asarone in Cisplatin induced hepatoxicity in experimental animals. Cisplatin is widely used as anticancer agent but it has main side effect like organ toxicity such as hepatotoxicity, nephrotoxicity etc. due to this side effect it used as experimental model for preclinical screening of drug or chemical as hepatoprotective.[19,20] In present study Cisplatin used as induced hepatotoxicty in rats at the dose of 7 mg/kg. Meanwhile

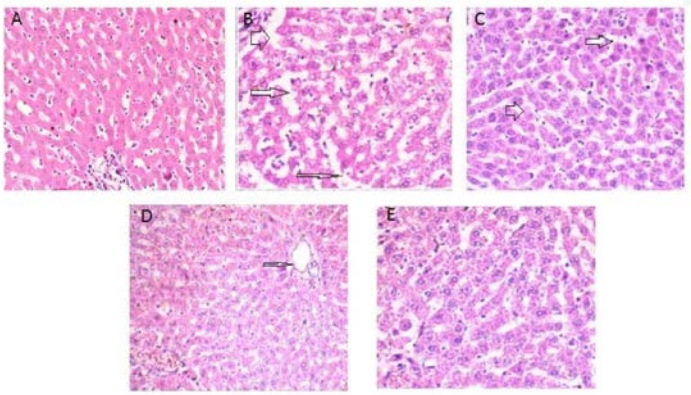

Figure 6: Effect of α- Asarone on histopathology of liver.
(A- Normal, B- Cisplatin, C- A100, D-A150, E-200)

α-asarone screened from hepatoprotective activity. α-asarone reported with several pharmacological activities.

In this study, we found that body weight of Cisplatin treated rats shows reduction in body weight and meanwhile treatment with α-asarone increases the body weight of the rats. In case of liver weight, it was increased in the Cisplatin treated rats and maintained in the α-asarone treated rats. The study showed that at the end of study level of serum ALT, AST, TB and HP were increased. However the rats treated with α-asarone were shows the normal level of all these as compared to the normal.

Oxidative stress refers a situation of a marked imbalance between the production and removal of ROS. This may be originated by an overproduction of these substances or by the depletion of antioxidant defenses this occurred due to Cisplatin toxicity.[4,12] Cisplatin treated rats shows oxidative damage in the rats, while rats treated with α-asarone shows the reverse effect on the oxidative damage. Imbalance of nitric oxide level found in the hepatoxicity, in present study Cisplatin treated rats shows increased level of nitric oxide and rats treated with α-asarone shows normal level of nitric oxide. Cytokine level increased in the rats treated with cisplatin[21] meanwhile α- Asarone treated rat's shows reduction the level of cytokine. As per the above observations, it was concluded that α-asarone shows protective effect against cisplatin induced hepatotoxicty in rats with normalizing the biochemical markers, maintaining the level of antioxidant enzyme and cytokines, protection of liver tissue from damage. In further studies we are going to conduct experiment on cell line for investigation of mechanism of α-asarone in protection of hepatotoxicty.

CONCLUSION

L-carnitine is having protective role in the bile duct ligation in rats. It reduces oxidative and nitrosative stress as well as reduced the concentration of release of inflammatory cytokines. It reduced the MRI score in the rats with BDL; hence, we consider that further studies are needed to clarify the possible role of L-carnitine in the liver toxicities.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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