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Hepatoprotective Effect Of *Allium Sativum* And *Acarus Calamus* Extract In Cisplatin Induced Hepatotoxicity In Experimental Rats

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Abstract

The purpose of this research was to determine whether or not allium sativum and acarus calamus had a protective effect against the hepatotoxic effects of cisplatin when administered to experimental rats. The methods included randomly assigning rats to one of five different groups. Cisplatin was administered at a dosage of 7.5 mg/kg, which resulted in the induction of hepatotoxicity. Serum levels of AST, ALT, total bilirubin, and albumin, as well as hepatic hydroxy proline (HP), reduced glutathione (GSH), and malondialdehyde (MDA), cytokines, and NO were assessed. The findings of this research demonstrated that therapy with allium sativum and acarus calamus led to normal body and liver weights, but treatment with cisplatin led to reduced body weight and increased liver weight in rats. Cisplatin-treated rats exhibited elevated levels of serum AST, ALT, total bilirubin, HP, GSH, MDA, and cytokines. The administration of allium sativum and acarus calamus to rats resulted in a decrease in the oxidative stress, an inhibition of the production of cytokines in a dose-dependent manner, and protection against hepatotoxicity. Allium sativum and acarus calamus were shown to have a protective effect against the hepatotoxicity caused by cisplatin, as was concluded by the research.

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Keywords: Cisplatin; Hepatotoxicty; ALT; oxidative stress; α- Asarone; Cytokine.

Introduction

Cisplatin is an anti-neoplastic medication that is used in the treatment of metastatic malignancies in addition to a number of other solid tumors. Although greater dosages of cisplatin are more efficient in suppressing the growth of cancerous cells, these doses also cause irreparable damage to the liver in addition to other adverse effects on organs [1]. Particularly when administered in large dosages, there is a possibility that the medication may accumulate to an excessive degree in the hepatic tissue. Cisplatin has some impressive anticancer qualities, but its clinical usage is often restricted due to the significant toxic side effects that it causes. These side effects interfere with the drug's therapeutic effectiveness [2, 3]. Damage to the liver was produced by cisplatin, most likely as a consequence of an elevated expression of CYP2E1, which is a potent generator of reactive oxygen

species. It has been shown that low doses of cisplatin that are administered frequently might produce hepatotoxicity, most likely as a result of liver accumulation [4, 5]. Nitrosative stress is associated with hepatotoxicity and is caused by reactive oxygen and nitrogen species (RNS) such as ONOO-, peroxinitrous acid (HONOO-), nitrogen dioxide radical (NO2*), and other species. RNS is created from NO*, which is produced by a series of NOS enzymes that regulate the manufacture of NO*[6]. RNS may then be further broken down into its component parts. There is a large range of pharmacological effects associated with the extract of allium sativum and acarus calamus. It has been demonstrated to have a wide range of pharmacological effects, including antioxidative, antihyperlipidemic, anti-inflammatory, antidepressant, antiepileptic, antianxiety, and anti-diabetic properties. These effects are caused by the interaction of allium sativum and acarus calamus extract with a variety of different molecular targets. Researchers have synthesized and examined a variety of isomers derived from acarus calamus and allium sativum extracts due to the antiplatelet and hypolipidemic effects that these compounds exhibit. The purpose of this research was to investigate whether or not allium sativum and acarus calamus had a protective effect against the hepatotoxicity caused by cisplatin in rats.

Material 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 (22-24^0 C) and relative humidity 65% with12 hour’s 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/2023/1-14

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(n=6)	Treatment
Normal	Each animals receives 1mlvehicle
Control	Received cisplatin (7.5mg/kg.) On 2nd day of study
AS 150	Allium sativum (150mg/kg/day) +cisplatin (7.5mg/kg.) On 2nd day of study
AC 150	Acorus calamus extract (150 mg/kg/day) +cisplatin (7.5mg/kg.) On 2nd day of study
AS+AC (150+150)	*Allium sativum and acarus calamus extract* (150+150 mg/kg/day) +cisplatin (7.5mg/kg.) On 2nd day of study

Collection of blood samples

The rats were put under with a dose of light ether anesthesia before being examined. The blood from each animal was collected in eppendorf tubes using the retro-orbital technique, and then the serum was separated by centrifugation using a cooled centrifuge at a speed of 10,000 revolutions per minute for ten minutes. The serum samples were kept at a temperature of -20 degrees Celsius until further examination.

Experimental parameters

On the first and tenth days of the experiment, the body weight of each group rat was determined using a weighing scale. At the conclusion of the study, the weight of each rat's liver was determined after scarification using a high dose of ether anesthesia. After removing the liver from each rat and washing it in phosphate buffered saline that had been chilled in the freezer, the livers were blotted on filter paper, weighed, and then homogenized.

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 most significant indication of membrane lipid peroxidation is the quantity of malondialdehyde, often known as MDA. This indicator may be found in liver tissues and can be measured using a technique that has been previously described. The process of lipid peroxidation was characterized by the creation of a pink color as a result of an interaction between MDA and thiobarbituric acid. The absorbance of a pink color was measured spectrophotometrically at a wavelength of 532 nm [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 150 + 150 mM/1 of sodium carbonate, 1 ml of 0.3% Triton X-150, 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].

Determination of Nitric oxide
Supernatant from homogenate (150 + 150 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 ($NaNO_2$) 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].

Result

Effect of allium sativum and acarus calamus on body weight and liver weight
The disease control group saw a gradual rise in body weight during the therapy periods, in contrast to the normal groups. The pattern of a gain in body weight is the same in both the normal group and the group that was treated with Allium sativum and acarus calamus at a dose of 150 mg/kg each. When compared to the control group, the administration of allium sativum and acarus calamus at a dose of 150 mg/kg had no impact on the subjects' body weight (P 0.005). At the conclusion of the research project, the weight of the animals' livers was determined, and our findings revealed a significant rise in the control group's liver weight in comparison to the normal group (P 0.005). In comparison to the control group, the allium sativum and acarus calamus treatment with 150 mg/kg of each demonstrated the most significant impact on the liver weight (P 0.005).

Table 1: Effect of allium sativum and acarus calamus on body weight and liver weight

Group	Body weight (gm)		Liver weight (gm)
	0 day	28 day	
Normal	1150.6± 5.63	186.3±8.39	5.65 ± 0.5
Control	176.5 ± 4.3	168.7±7.16	6.03 ± 1.6###
As 150	181.2±5.36	186.3±7.52	5.49 ± 1.1
Ac 150	176.4±5.63	188.5±5.37	5.03 ± 0.8**
As+ac (150+150)	172.8±6.85	183.6.4±6.38	5.65 ± 0.7***

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.

Effect of allium sativum and acarus calamus on biochemical parameters

The current investigation revealed that there was a statistically significant rise in the levels of serum AST, ALP, ALT, total bilirubin, and HP in the control group as compared to normal rats (P 0.001) in all of the aforementioned biomarkers. The level of biochemical markers in rats treated with a dose of 150 mg/kg allium sativum and acarus calamus had no effect, but rats treated with doses of 75 and 75 mg/kg allium sativum and acarus calamus showed a decrease in level of biochemical markers as compared to the control group (P 0.005) as shown in figures 1 and 2. The toxicity of the liver in rats was demonstrated by an increase in biochemical levels in the animals in the control group. In the group that was just given allium sativum and acarus calamus at a dose of 150 mg/kg, there was no discernible change in the levels of biochemical markers.

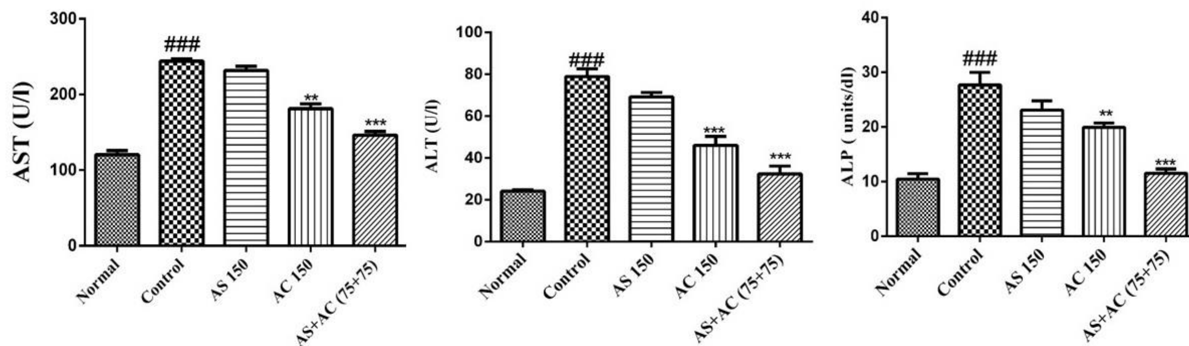

Figure1. Effect of allium sativum and acarus calamus on biochemical parameters

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

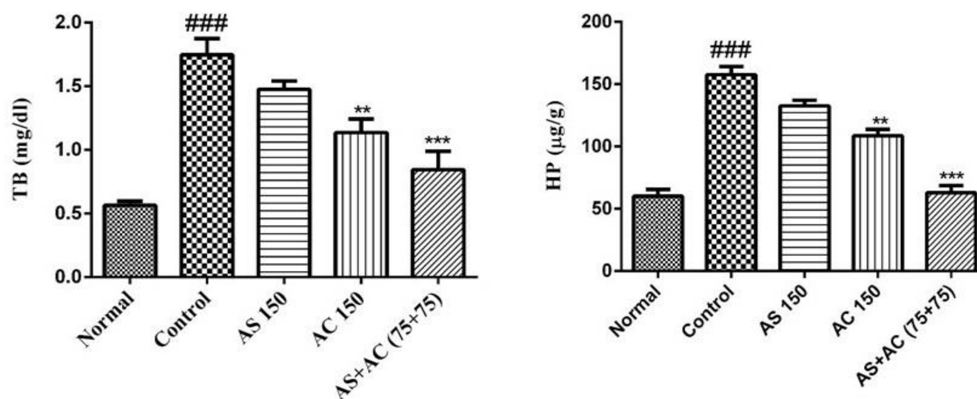

Figure2. Effect of allium sativum and acarus calamus on biochemical parameters

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

Effect of allium sativum and acarus calamus on serum cytokine

A increase in the pro-inflammatory cytokine production of IL-6, IL-1, and TNF- in the blood was used to evaluate the impact of both allium sativum and acarus calamus. At the conclusion of the trial, it was discovered that the control group had a much higher level of the pro-inflammatory cytokines IL-6, IL-1, and TNF- when compared to the normal group. Treatment with allium sativum and acarus calamus (75 and 75 mg/kg) for ten days dramatically lowered the level of IL-6, IL-1, and TNF- levels practically to the normal levels, which indicates that allium sativum and acarus calamus has an inhibitory impact on the production of cytokines. When compared to the normal group, the level of IL-10 was found to be significantly higher in the control group. A dose-dependent impact was seen in rats that were given allium sativum and acarus calamus as compared to the rats in the control group, as shown in figure 3.

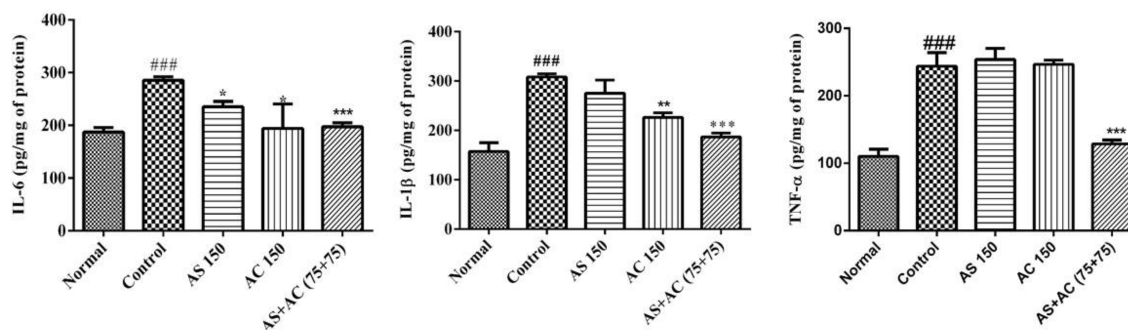

Figure 3. Effect of allium sativum and acarus calamus on cytokine level

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

Effect of Allium sativum and acarus calamus on oxidative stress

In rats with cisplatin-induced liver fibrosis, levels of both SOD and GSH were significantly lower than those seen in the normal group. When compared with the group that was treated with cisplatin, the group that was given treatment with allium sativum and acarus calamus (75 mg/kg) for 10 days exhibited elevated levels of SOD and GSH. A lower amount of glutathione peroxidase (GSH) was found in the Cisplatin-induced liver damage group in comparison to the Normal group. When compared with the disease control group, the results of an oral therapy with allium sativum and acarus calamus (75 and 75 mg/kg) for ten days reveal a lower level of MDA. When compared to the control group, the allium sativum and acarus calamus treatments at doses of 75 and 75 mg/kg respectively demonstrated the most notable impact on the MDA level. A lower level of catalase activity was seen in the Cisplatin-Induced Liver Toxicity group compared to the Normal group. Oral therapy with allium sativum and acarus calamus (75 and 75 mg/kg) for ten days demonstrates a considerably higher level of catalase activity in comparison to the Cisplatin-treated group, as seen in figure 4.

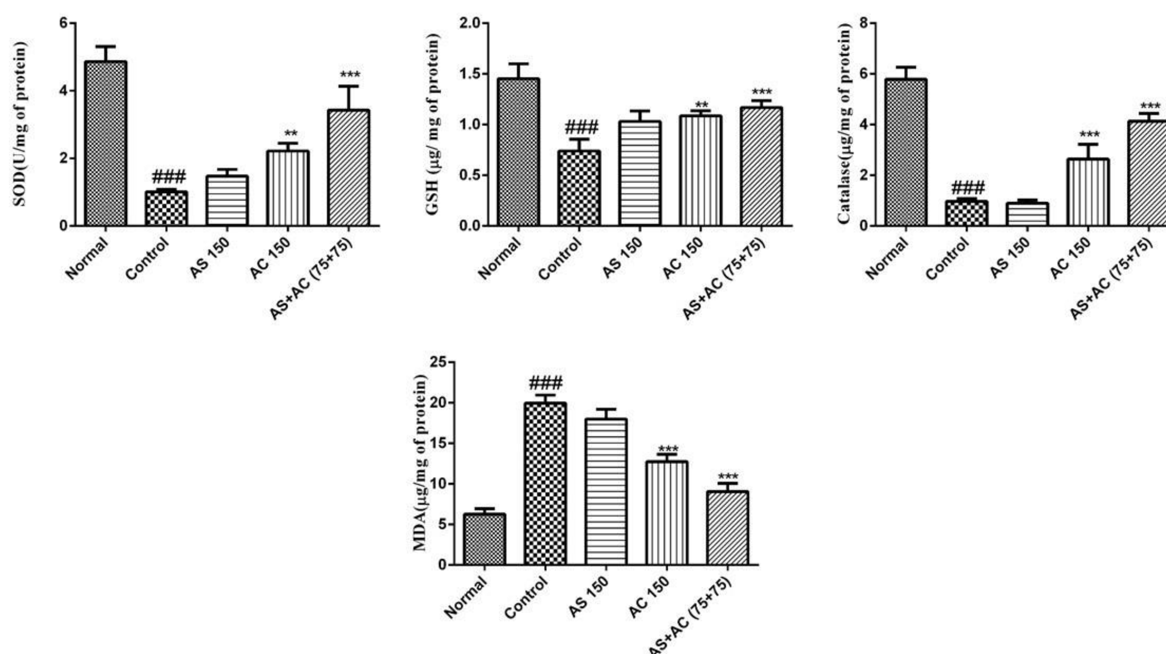

Figure 4. Effect of allium sativum and acarus calamus on oxidative stress

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

Effect of allium sativum and acarus calamus 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 allium sativum and acarus calamus with different dose shows decrease in the level of nitric oxide dose dependently. Rats treated with allium sativum and acarus calamus 75 and 75 mg/kg shows significant decrease in the level of nitric oxide as compared to Cisplatin treated rats shown in figure 5.

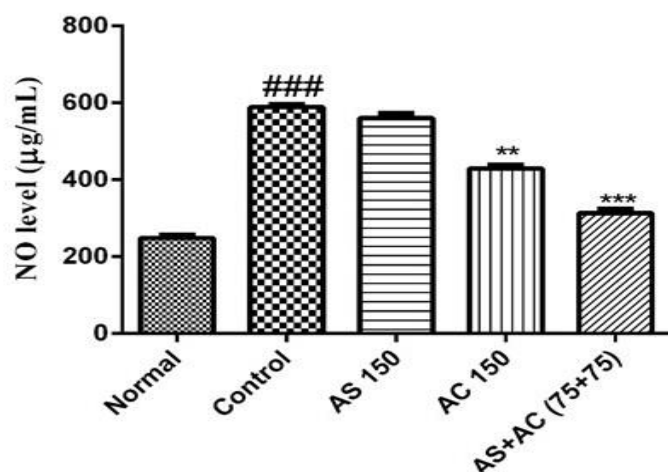

Figure 5- Effect of allium sativum and acarus calamus on nitric oxide

Discussion

A condition in which there is a significant imbalance between the generation of ROS and their elimination is referred to as oxidative stress. This may have been caused by an excessive synthesis of these compounds or by the depletion of antioxidant defenses that took place as a result of the toxicity caused by cisplatin[4, 12]. The treatment of rats with cisplatin results in oxidative damage in the rats, but the treatment of rats with allium sativum and acarus calamus results in the opposite impact on the oxidative damage. In the current research, rats treated with cisplatin exhibit higher levels of nitric oxide, but rats treated with allium sativum and acarus calamus show normal levels of nitric oxide. This indicates an imbalance in the levels of nitric oxide, which was detected in the hepatotoxicity. The level of cytokine was found to be higher in the rats that had been treated with cisplatin [21], but the level of cytokine was found to be lower in rats treated with allium sativum and acarus calamus. It was determined that allium sativum and acarus calamus has protective effect against cisplatin induced hepatotoxicty in rats with normalizing the biochemical indicators, maintaining the level of antioxidant enzyme and cytokines, and protecting liver tissue from damage. These findings were based on the results that were presented before. In the next phase of our research, we want to perform an experiment on a cell line in order to investigate the mechanism of action behind the protective effects of allium sativum and acarus calamus against hepatotoxicity.

Conclusion

Both allium sativum and acarus calamus have been shown to have a protective effect against hepatotoxicity. It does this by lowering the levels of oxidative and nitrosative stress, as well as the concentration of inflammatory cytokines that are released.

CONFLICT OF INTERESTS

All authors have no conflicts of interest to declare.

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