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Protective Role of *Cocos nucifera* Linn against Ethanol Induced Hepatotoxicity in Wistar Rats

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ABSTRACT

Alcoholic liver disease (ALD) is a major health problem worldwide. ALD typically progresses through the stages of alcoholic steatosis, alcoholic hepatitis, and alcoholic cirrhosis. Herbal drugs play a role in the treatment and management of various liver disorders. The current study was aimed at evaluating the protective role of *Cocos nucifera* Linn administered at two doses (200 mg/kg & 400 mg/kg, b.wt., p.o) and silymarin (50 mg/kg, b.wt., p.o) for 7 consecutive days against 50% v/v ethanol (3.75 gm/kg, b.wt., i.p) induced hepatotoxicity in wistar rats. It has been observed that pretreatment with hydro alcoholic extract of roots of *Cocos nucifera* Linn (HACN) & Silymarin could significantly minimize the toxicity induced by ethanol as evidenced by significant ($P<0.0001$) restoration of altered plasma biochemical markers SGOT, SGPT, ALP and Total protein. In addition, pretreatment with HACN and silymarin resulted in significant increase in SOD, CAT enzymes and reduced MDA levels as evidenced by the histopathological observation of liver preserving the histoarchitecture of the tissue to near normal. These results suggest that biologically active phytoconstituents present in the *Cocos nucifera* Linn may be responsible for the significant hepatoprotective and anti-oxidant activity.

INTRODUCTION

Liver diseases has become one of the major cause for morbidity and mortality in humans all over globe and hepatotoxicity due to drug appears to be the most common contributing factor [1]. Drug induced liver injury accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (39%) and idiosyncratic liver injury triggered by other drugs (13%) [2]. Excess consumption of certain drugs like antibiotics, chemotherapeutic agents, acetaminophen and exposure to some chemicals such as peroxidised oils, Aflatoxins, Carbon tetrachloride (CCl_4), Alcohol consumption etc. results in free radical generation makes liver more vulnerable to variety of disorders viz., jaundice, hepatitis etc. which are the two major hepatic disorders that account for high death rate [3]. Therefore, many folk remedies from plant origin are being tested for their potential antioxidant and hepatoprotective property in experimental animal models [4-5].

Cocos nucifera Linn (Family: Palmae) commonly referred as Coconut or Nariel. Its bark is smooth and grey, marked by ringed scars left by fallen leaf bases. Unlike some other plants, the palm tree does not have tap root hairs but has fibrous root system. The activities of the root include astringent, dentifrice; decoction of root promotes flow of urine and is used in the diseases of the uterus, bronchitis and dysentery. It has antihelminthic activity and anti-bacterial agent, in treatment for urinary tract infections and also in some skin infection [6-7]. It also posses wound healing property [8]. The phytochemical investigation of the root extract has explored the presence of flavonoids, glycosides, tannins, terpenoids, saponins, carbohydrates [8].

The present study was undertaken to investigate the protective role of hydro alcoholic extract of *Cocos nucifera* Linn (HACN) against ethanol induced hepatotoxicity model.

MATERIALS AND METHODS

Collection and Authentication of plant material

The roots of *Cocos nucifera* Linn were collected from surrounding areas of Tirupati, Chittoor district of Andhra Pradesh region and was authenticated (Voucher No.2547) by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V University, Tirupati, Andhra Pradesh, India.

Preparation of test extract

The roots were washed under running tap water, cut into small pieces of 2-3cm and shade dried (36ºC, 50 ± 5% relative humidity) for 15 days. The shade dried plant material was powdered using pulverizer to get coarse powder. The coarsely powdered root of *Cocos nucifera* Linn was used for the extraction with hydro alcoholic solvent (70%) in 1:4 volumes by cold maceration in a narrow mouthed bottle for seven days with occasional shaking [9]. After completion of extraction, the solvent was filtered and evaporated for obtaining dry residue. The residue was then weighed and yield was recorded. Preliminary phytochemical screening of HACN was carried out for the identification of the various bioactive constituents [10].

Experimental animals

Healthy male wistar rats weighing about 150-200 gms were procured from animal house of Sri Venkateswara enterprises, Bangalore and were acclimatized to a standardized environmental conditions (temperature 25 ± 2º C and 12 hrs light/12 hrs dark cycle) of animal house, department of Pharmacology, Seven Hills College of Pharmacy, Tirupati. The animals were given commercial pellet diet ad *libtium* and had free access to water. The experimental protocol was approved by Institutional Animal Ethical Committee of Seven Hills College of Pharmacy, Tirupati (Registered No. 1995/PO/Re/S/17/CPCSEA).

Experimental design:

Animals were divided into five groups each containing six animals. Group I animals served as vehicle control and received 0.5 ml of normal saline orally (p.o) throughout the study period. Group II animals served as hepatotoxic and received with a modified dose of 3.75 gm/kg of 50% v/v ethanol administered intraperitoneally (i.p) daily for 7 consecutive days [11]. Group III animals served as standard control received Silymarin (50 mg/kg, b.wt., p.o) daily for 7 days and treated with 3.75 gm/kg of 50% v/v ethanol administered i.p 2 hours after the pretreatment with silymarin. Group IV & V animals served as test control received HACN at graded doses of 200 mg/kg, b.wt. & 400 mg/kg, b.wt. orally daily for 7 days and treated with 3.75 gm/kg of 50% v/v ethanol administered i.p 2 hours after the pretreatment with HACN.

Biochemical and Histopathological Estimations

After the study period, the animals were euthanized and the blood was collected through cardiac puncture, the collected blood was centrifuged at 4000 rpm for 15 min and plasma was separated for biochemical marker analysis includes SGOT, SGPT**,** ALP and Total Proteins [12-14]. The liver tissues were isolated and estimated for pro-oxidant and antioxidant parameters [15-17] and histopathological observations [18].

Statistical analysis

The results were expressed as Mean ± SEM and analyzed statistically using one way ANOVA followed by Dunnett's test. Data's were computed for statistical analysis using the Prism graph pad software version 5.

RESULTS

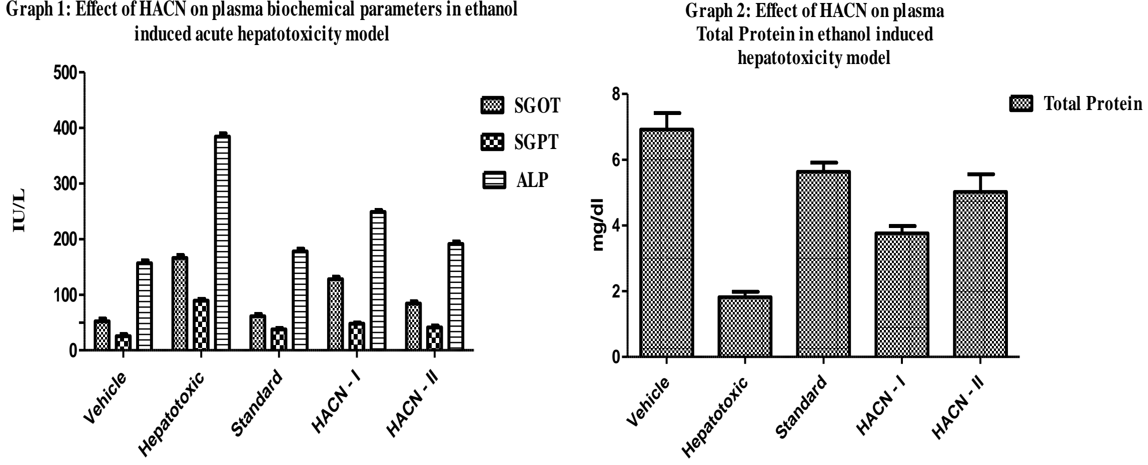
Effect of pharmacological interventions on biochemical marker profile

Table 1 shows a significant increase (P<0.001) in plasma biochemical parameters SGOT, SGPT, ALP and decrease in plasma total proteins in ethanol induced hepatotoxicity model when compared to normal vehicle control. Treatment with HACN at 200 & 400 mg/kg b.wt. ameliorated the altered plasma biochemical parameters compared to hepatotoxicity induced group.

Table 1: Effect of HACN on plasma biochemical parameters in ethanol induced hepatotoxicity

Control groups	SGOT [IU/L]	SGPT [IU/L]	ALP [IU/L]	TP [gm/dL]
Vehicle [0.5 mL of saline, p.o]	52.4 ± 4.46	46.42 ± 3.37	156.8 ± 4.96	6.92 ± 1.24
Hepatotoxic [3.75 gm/kg of 50% v/v ethanol, i.p]	165.9 ± 5.22	189.22 ± 2.98	384.5 ± 5.58	1.82 ± 0.88
Standard [Silymarin-50 mg/kg, b.wt., p.o]	61.2 ± 4.13***	57.58 ± 2.45***	177.8 ± 4.75***	5.63 ± 0.283***
HACN – I [200 mg/kg, b.wt., p.o]	127.7 ± 4.53**	107.84 ± 1.96**	248.7 ± 3.81**	3.76 ± 1.042**
HACN – II [400 mg/kg, b.wt., p.o]	84.2 ± 3.86***	73.1 ± 3.2***	191.2 ± 4.30***	5.02 ± 0.54***

Values were expressed as Mean ± SEM values (n=6). Comparison was made by One Way ANOVA by Dunnett's multiple comparison test at significance value ***P<0.0001, **P<0.001, *P<0.05 indicates comparison with hepatotoxicity control group.



Effect of pharmacological interventions on tissue parameters

Table 2 shows a significant increase (P<0.001) in MDA levels with decrease (P<0.001) in tissue antioxidant enzymes SOD and CAT in ethanol induced hepatotoxicity model compared to normal vehicle control. The treatment with graded doses of HACN showed reduction in MDA levels with subsequent increase in SOD and CAT as compared to the hepatotoxic model.

Table 2: Effect of HACN on liver parameters in ethanol induced hepatotoxicity

Control groups	CAT [µmoles of H_2O_2/min/mg protein]	SOD [U/mg protein]	MDA [µM of MDA/mg of wet liver tissue]
Vehicle [0.5 mL of saline, p.o]	4.38 ± 0.60	5.20 ± 1.34	5.50 ± 1.19
Hepatotoxic [3.75 gm/kg of 50% v/v ethanol, i.p]	2.16 ± 0.45	2.63 ± 0.74	13.44 ± 1.52
Standard [Silymarin-50 mg/kg, b.wt., p.o]	4.16 ± 0.68***	4.81 ± 0.51***	5.72 ± 1.05***
HACN – I [200 mg/kg, b.wt., p.o]	3.16 ± 0.62*	3.24 ± 0.58**	9.07 ± 1.58**
HACN – II [400 mg/kg, b.wt., p.o]	3.79 ± 0.81***	4.12 ± 0.54***	7.19 ± 1.73***

Values were expressed as Mean ± SEM values (n=6). Comparison was made by One Way ANOVA by Dunnett's multiple comparison test at significance value ***P<0.0001, **P<0.001, *P<0.05 indicates comparison with hepatotoxicity control group.

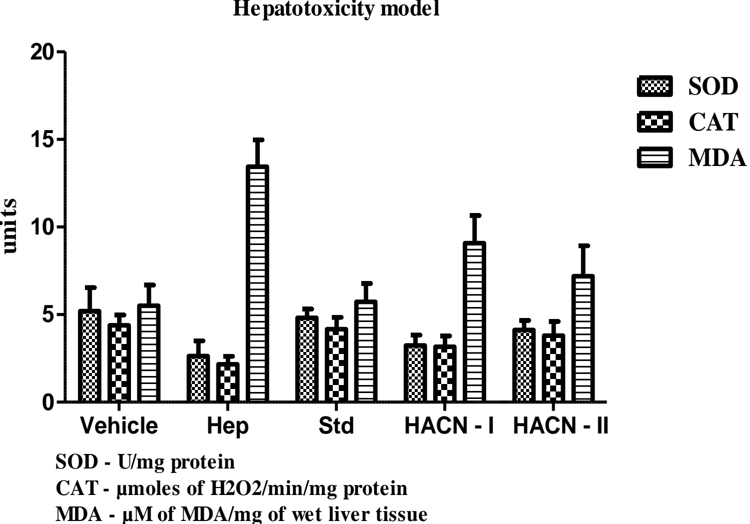
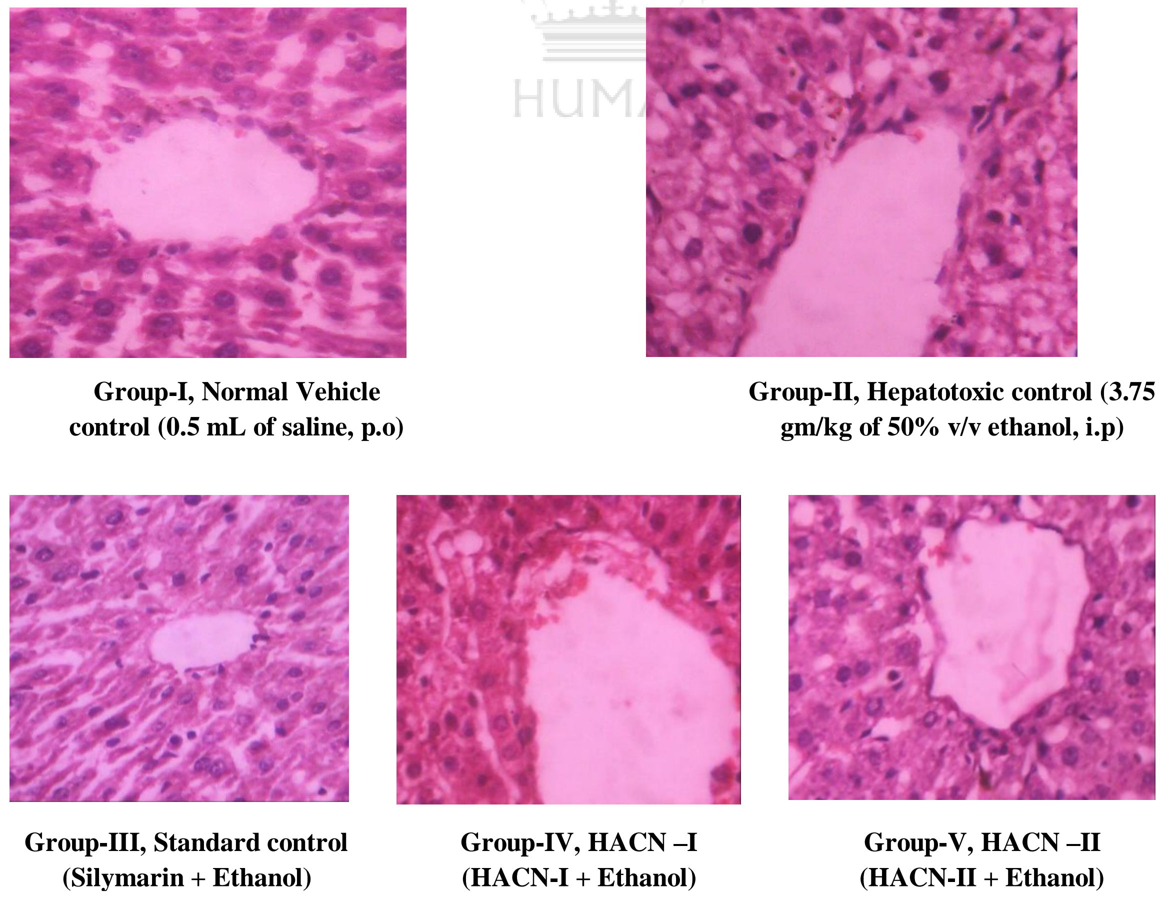


Figure 1: Histopathology of Liver tissue



Group-I, Normal Vehicle control (0.5 mL of saline, p.o)

Group-II, Hepatotoxic control (3.75 gm/kg of 50% v/v ethanol, i.p)

Group-III, Standard control (Silymarin + Ethanol)

Group-IV, HACN –I (HACN-I + Ethanol)

Group-V, HACN –II (HACN-II + Ethanol)

DISCUSSION

Hepatotoxicity is one of very common aliment resulting into serious debilities ranging from severe metabolic disorders to even mortality [19]. Ethanol is hepatotoxic through redox changes produced by the NADH generated in its oxidation via the alcohol dehydrogenase pathway, which in turn affects the metabolism of lipids, carbohydrates, proteins and purines [20]. About 80% of ingested alcohol is metabolized to the cytotoxic acetaldehyde by the enzyme alcohol dehydrogenase in the liver. It is well known aldehyde oxidase or xanthine oxidase oxidizes acetaldehyde to acetate, forming reactive oxygen species (ROS) through liver metabolic enzyme cytochrome P450 2E1 (CYP 2E1) [21].

Previous reports demonstrates that chronic alcohol consumption induces oxidative stress by multiple mechanisms [22, 23] and a variety of pathological conditions ranging from simple intoxication to severe life threatening pathological states [24]. In liver, two major enzyme systems involved in the metabolism of alcohol i.e. alcohol dehydrogenase and microsomal ethanol oxidizing system (MEOS). Alcohol dehydrogenase converts alcohol to acetaldehyde by removing hydrogen. Then second enzyme, aldehyde dehydrogenase in hepatic mitochondria, oxidizes acetaldehyde to acetate by removing additional hydrogen and adding oxygen. Acetaldehyde depletes glutathione levels which impairs major defense mechanism against oxidative damage promoting cell death. Lipid peroxidation results in the formation of more free radicals which can further damage cell and organelle membrane causing more liver cell injury inducing oxidative damage [25].

In current study, Silymarin was used as a standard drug, a naturally occurring polyphenolic flavonoid isolated from *Silybum marianum* used medicinally for centuries as an herbal medicine in various liver related disorders. Silymarin offers hepatoprotective effect against hepatotoxins through various mechanisms such as anti-oxidant activity, inhibition of lipid peroxidation etc. [26-28].

Upon ethanol administration, the concentrations of cellular enzymes like SGOT, SGPT and ALP present in the liver cells leak into the plasma during liver damage [29-33]. Elevated activities of these enzymes in plasma indicate hepatocytes damage and leakage of cell membrane [34, 35]. Ethanol administration decreased the levels of total protein attributed to the higher concentration of alcohol dehydrogenase enzyme catalyses alcohol to aldehyde and accumulation of export type proteins due to inhibition of their secretion from the liver in

alcoholics [36, 37]. Pretreatment with silymarin & HACN at 200 & 400 mg/kg, b.wt., ameliorated the altered plasma biochemical parameters as compared to hepatotoxic group which was depicted in Table 1 and Graph 1 & 2.

Oxidative stress is a serious causative factor for hepatic dysfunction and plays a very important role in pathophysiology of several diseases and is an imbalance between pro-oxidants and antioxidants. Induction of oxidative stress was identified as principal element in pathophysiology of liver injury induced by alcohol administration [38]. In this study, induction of ethanol provokes free radical attack on membrane lipids resulting in elevation of malondialdehyde (MDA), with subsequent reduction in SOD and CAT enzyme levels [39]. Pretreatment with HACN at doses 200 & 400 mg/kg, b.wt., showed reduced levels of MDA and increased levels of SOD and CAT enzymes which was shown in table 2 & graph 3 respectively. The results reflected may have beneficial and reducing risk factors for hepatotoxicity.

Histopathological observation of liver in vehicle control animals showed normal architecture, whereas the liver section of animals treated with ethanol showed distorted liver architecture with hepatocytes showing degenerative changes and necrosis [40-41]. The liver section of animals treated with silymarin and HACN showed significant restoration of hepatocytes architecture compared with hepatotoxicity induced group.

Investigation on various plant phytochemicals revealed the presence of flavonoids and phenolics has the potential free radical scavenging activity [42]. Flavonoids and phenolic compounds have been reported to exhibit multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory activity, anticarcinogenic activity etc. for the prevention and cure of various diseases which is mainly associated with free radicals. [43] The phytochemicals like phenols, flavonoids, and tannins may be the reason for the hepatoprotective activity through which the antioxidant enzymes may be raised and oxidative stress may be plugged.

CONCLUSION

The herbal medicine is gaining more importance because of their less toxic nature. Hence there is a need to identify and develop natural antioxidants from plant sources to replace synthetic antioxidants for long term safety. The findings of the above study suggest that HACN was proved for its hepatoprotective effect which significantly ameliorated the altered

plasma biochemical, tissue prooxidant and anti-oxidant parameters in ethanol induced hepatotoxicity in Wistar rats as evidenced by the histopathological examination of liver. Therefore, it can be recommended for further investigation on the isolation of the compound to reveal the exact mechanism for hepatoprotective effect exhibiting anti-oxidant property.

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