Research Article

Hepatoprotective Effect of *Allamanda neriifolia Hook* on 1, 4 Dichlorobenzene induced Hepatotoxicity in Rats.

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ABSTRACT

A large number of populations suffer due to various reasons from hepatic diseases of unknown origin. The development of antihepatotoxic drugs being a major thrust area has drawn the attention of workers in the field of natural product research because synthetic drugs may cause serious side effect. Methanol extract of flowers of *Allamanda neriifolia hook* was evaluated for hepatoprotective and antioxidant activities in rats. The plant extract (100 and 300 mg/kg, p.o.) showed a remarkable hepatoprotective and antioxidant activity against 1,4 Dichlorobenzene (DCB) induced hepatotoxicity as judged from the serum marker enzymes and antioxidant levels in liver tissues. 1,4 DCB induced a significant rise in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin, lipid peroxidase (LPO) with a reduction of total protein, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione S-transferase (GST). Treatment of rats with different doses of plant extract (100 and 300 mg/kg) significantly ($P<0.001$) altered serum marker enzymes and antioxidant levels to near normal against 1,4 DCB treated rats. The activity of the extract at dose of 300 mg/kg was comparable to the standard drug, silymarin (100 mg/kg, p.o.) The biochemical observations were supplemented with histopathological examination of rat liver sections. The present study revealed that *Allamanda neriifolia hook* of high dosage possess a significant hepatoprotective and antioxidant activity and it can be employed in protecting tissue from oxidative stress.

Keywords: *Allamanda neriifolia hook,* 1,4 Dichlorobenzene(DCB) , antioxidant enzymes. Hepatoprtective activity.

INTRODUCTION

Liver is the key organ for detoxication and disposition of endogenous substances. It is continuously and widely exposed to xenobiotics, hepatotoxins, and chemotherapeutic agents that lead to impairment of its functions.[1] Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages.[2]

Hepatotoxicity is one of very common aliment resulting into serious debilities ranging from severe metabolic disorders to even mortality. Hepatotoxicity in most cases is due to free radical. Free radicals are fundamental to many biochemical processes and represent an essential part of aerobic life and metabolism.[3] Reactive oxygen species mediated oxidative damage to macromolecules such as lipids, proteins and DNA has been implicated in the pathogenecity of major diseases like cancer, rheumatoid arthritis, degeneration process of aging and cardiovascular disease etc. Antioxidants have been reported to prevent oxidative damage caused by free radicals by interfering with the oxidation process through radical scavenging and chelating metal ions.[4]

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effect. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.[5] Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc.[6]

Plant extracts of many crude drugs are also used for the treatment of liver disorders. Extracts of 25 different plants have been reported to cure liver disorders.[7] *Allamanda neriifolia hook*, locally known as yellow bell belongs to the family *Apocynaceae,* commonly known as oleander or dogbane family with about 200 genera and 2,000 species are most commonly found in tropical and subtropical regions and have an ornamental value.

In view of this, the present study was aimed at evaluating the hepatoprotective activity of methanolic extract of flowers of *Allamanda Neriifolia Hook against* 1, 4 Dichlorobenzene induced hepatotoxicity in albino rats.

MATERIALS AND METHODS

Plant material

Flowers of *A.neriifolia* was collected from Bharathidasan University Tiruchirappalli. The Plant was identified by Dr. S. John Britto, The Director, The RAPINAT Herbarium and

Centre for Molecular Systematic, in St. Joseph's College, Tiruchirappalli.

Preparation of Extracts

The flowers of *A.neriifolia* was collected and shade dried. The dried flowers were coarse powdered and the powder was packed in to sox let column and extracted with methanol (65.5°C).

The extract was concentrated under reduced pressure (bath temp 50°C). The dried extracts were stored in air tight container.

Animals

Male albino rats of Wistar strain approximately weighing 100-150g were used in this study.

They were healthy animals Purchased from the Indian Institute of Science, Bangalore.

The Animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2°C and 12 hour light/dark cycle) throughout the experimental period.

All the animals were fed with standard pellet diet and water were provide ad libitum. They were acclimatized to the environment for one week prior to experimental use. Before starting the experiment, permission from the Institutional Animal Ethics Committee was obtained. IAEC No: 02/003/2014.

Reagents and Chemicals

1,4 Dichloro benzene, TBA, DNPH like all chemicals were of analytical grades and chemicals required for sensitive biochemical assay were obtained from" M/S sigma and Aldrich chemical co., U.S.A.," Double distilled water was used in all biochemical assays.

Experimental Design

1, 4 DCB is used to induce liver toxicity in albino rats. The rats were assorted into the following group.

Group I : (n=6) – Negative control rats. Diet and water were available ad libitum.

Group II : (n=6) – 1, 4 DCB induced rats. 300mg of 1,4 DCB was dissolved in 1ml of corn oil & given per day for a period of 45 days.

Group III : 100mg/kg body weight Silymarin – [standard drug] was given after treating with 1, 4 DCB.

Group IV : (n=6) –*A.Neriifolia* flower extract treated rats. 100mg of *A. Neriifolia* flower extract/ kg/b.wt was given after treating with 1,4 DCB.

Group V : (n=6) –*A.Neriifolia flower* extract treated rats. 300mg of *A. Neriifolia* flower extract/kg/b.wt given after treating with 1,4 DCB.

Assessment of hepatoprotective activity

The blood was collected by Sino – orbital puncture and allowed to clot for few minutes, the Clotted blood was transformed to centrifuge tube. The blood was centrifuged at 3000rpm for 5min. The serum was used for the estimation of biochemical parameters like AST[8], ALT,[9] ALP[10], total bilirubin[11] and total protein.[12] After the collection of blood samples the rats in different groups were sacrificed and their livers were excised immediately and washed in ice cold normal Saline, followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A 10%w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation.[13] A part of homogenate after precipitating proteins with Trichloroacetic acid (TCA) was used for estimation of reduced glutathione and glutathione peroxidise.[14] The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 40°C. The supernatant thus obtained was used for the estimation of SOD[15] and CAT activities.[16]

Histopathiological Examination

Liver pieces were preserved in 10% formaldehyde solution. The pieces of liver processed and embedded in paraffin wax. Sections of about 4-6 microns were made and stained with haematoxylin and eosin and photographed.

Statistical Analysis

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Student' t test. P values < 0.05 were considered as significant.

RESULTS

Biochemical Parameters

The animals treated with1, 4 DCB exhibited a significant (P<0.001) rise in AST, ALT, ALP and total bilirubin levels when compared to the control group. This was significantly (P< 0.01) reduced after treatment with extract of flowers of *A.Neriifolia* which was almost similar to that of Silymarin [Table 1].

Total Protein

Total protein level was significantly (P< 0.001) reduced in 1, 4 DCB -treated group when compared to the control and was significantly elevated in the extract of flowers of *A.Neriifolia* treated groups. This was comparable to that of Silymarin treated group [Table 1].

Antioxidant Enzymes and Glutathione Levels

The levels of antioxidant enzymes such as SOD, CAT, GSH and GPx were decreased significantly (P< 0.001) in 1, 4 DCB administered rats and was significantly (*P*< 0.01) elevated in extract of flowers of *A. Neriifolia* treated group. This was comparable with that of Silymarin - treated group [Table 2]. Where as the 1, 4 DCB treatment rats were resulted in an increased the lipid peroxide levels in liver.

Administration of extract of flowers of *A.Neriifolia* prevents the accumulation of lipid peroxides. At a lower dose (100mg/kg/b.wt) of plant extract it was produced a marginal effect in all the parameters and at a higher dose (300mg/kg/b.wt) it can produce a better results compared to that of standard drug.

Table 1: Effect of methanolic extract *of A.Neriifolia* on Liver marker enzymes

Parameter	Group I	Group II	Group III	Group IV	Group V
AST(U/L)	21.19±1.9	85.4±5.5*	59.7±4.1#	63.04±5.2	43.5±3.1#
ALP(U/L)	38.9±2.6	81.3±2.8*	50.1±3.9#	68.9±5.8♣	48.3± 2.8#
ALP(IU/L)	74.9±4.2	187.6±10.7*	101.4±3.8#	180.9±4.1	99.4±5.3#
Total Bilirubin (gm/dl)	0.64±0.03	2.18±0.15*	1.30±0.08#	1.59±0.08♣	1.06±0.09#
Total Protein (mg/dl)	8.9±0.61	5.5±0.89*	7.5±0.87#	6.8±0.71	8.4±0.84#

Results are expressed as mean ±S.E.M, n=6. *P≤0.001, statistically significant as compared with control rats and ♣P≤0.01; #P≤0.001 statistically significant as compared with DCB induced group.

Table 2: Effect of methanolic extract of *A. Neriifolia* on Lipid peroxidative and antioxidant parameters

Parameter	Group I	Group II	Group III	Group IV	Group V
LPO	2.37±0.18	4.89±0.23*	3.09±0.22#	4.01±0.38	3.07±0.31#
SOD	7.41±5.5	3.85±0.19*	6.84±0.62#	5.18±0.47♣	6.35±0.45#
CAT	75.1±5.4	51.4±3.1*	66.5±0.61#	60.4±4.1♣	64.9±2.4#
GSH	46.7±1.5	23.9±1.2*	40.9±204#	35.1±2.7♣	38.2±1.7#
GPx	46.9±1.9	20.9±1.1*	39.8±2.6#	30.6±1.5♣	38.7±1.6#

Results are expressed as mean ±S.E.M, n=6. *P≤0.001, statistically significant as compared with control rats and ♣P≤0.01; #P≤0.001 statistically significant as compared with DCB induced group.

SOD-unit /min/mg protein; CAT-µmoles of H_2O_2 min/mg protein; GPx-µg of GSH consumed/in/mg protein; GSH-µ moles/g/ wet tissue; LPO- n moles of malondialdehyde/mg/protein.

Histopathology

The histopathological examination showed that treatment with caused typical centribular hepatocytic steatosis (both macro vesicular and micro vesicular) and necrosis, limiting plate necrosis, apoptosis, especially in the periportal hepatocytes and portal triaditis as compared with control liver. Liver tissues exposed to the extract of flowers of *A. Neriifolia* and Silymarin were almost similar to the control in histology, size and staining properties and showed only mild congestion. In the herbal formulation-treated group, there was a reduction in inflammation and it significantly prevented the degeneration of hepatocytes. Thus, histological examination clearly demonstrated the protection of liver against 1,4 DCB induced cytotoxicity.

DISCUSSION

1, 4 Dichlorobenzene (1, 4 DCB) has been used as a space deodorant and moth repellent as well as an intermediate in the chemical industry. Given its broad applications and high volatility, considerable concern exists regarding the adverse health effects of 1, 4 Dichlorobenzene in the home and the workplace. The primary exposure to 1, 4-dichlorobenzene is from breathing contaminated indoor air. Acute (short-term) exposure to 1, 4-dichlorobenzene, via inhalation in humans, results in irritation of the skin, throat, and eyes. Chronic (long-term) 1, 4-dichlorobenzene inhalation exposure in humans results in effects on the liver, skin, and central nervous system (CNS). When 1, 4 DCB was added to liver microsomes of rats epoxide formation resulted in considerable covalent binding to proteins.

In the assessment of liver damage by 1, 4 DCB the determination of enzyme levels was used. Serum AST, ALT, ALP and total bilirubin are the most sensitive markers used in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage. In this study, an increase in the activities of AST, ALT, ALP and total bilirubin in serum evidenced the1, 4 DCB induced hepatocellular damage.[17-20] The reduction of these enzyme levels in animals treated with the plant extract showed their ability to restore the normal functional status of the damaged liver.[19,20]

The determination of malondialdehyde (MDA) level is one of the most commonly used methods for monitoring lipid peroxidation.[18]

The result suggests that there was a dramatic increase in lipid peroxidation after 1, 4 DCB treatment and it was inhibited by the treatment with the extraction revealing that it exhibits potent hepatoprotective activity.

Measurement of total protein concentration was mainly used to calculate the level of purity of a protein. Maximum doses of1, 4 DCB causes the depletion of total proteins indicating tissue damage which was also evidenced in this study. Treatment with 1, 4 DCB significantly decreased SOD, CAT, GSH, and GPx stores indicating that they were used for the detoxification of toxic metabolites of the drug. The extraction restored the antioxidant enzyme levels significantly and reduced the 1, 4 DCB -induced oxidative injury, thus proving its antioxidant potential.[21]

The histopathological examination of the liver of the control group showed normal hepatocytes with portal triad [Figure 1].

The liver section of 1, 4 DCB treated rats showed typical centribular hepatocytic steatosis (both macrovesicular and microvesicular) and necrosis [Figure 2].

This could be due to the formation of highly reactive free radicals because of oxidative stress caused by1, 4 DCB. Simultaneous administration of herbal formulation prevented these effects [Figures 4 and 5].

Thus, histopathological studies revealed that concurrent administration of two different doses of the extraction exhibited protection of liver cells, which could be further confirmed the above results.

CONCLUSION

From the study, it can be concluded that the methanolic extract *of flowers of Allamanda Neriifolia Hook* showed a significant hepatoprotective and antioxidant activities on 1,4 DCB induced hepatic damage in rats.

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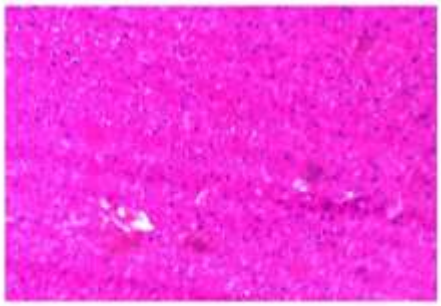
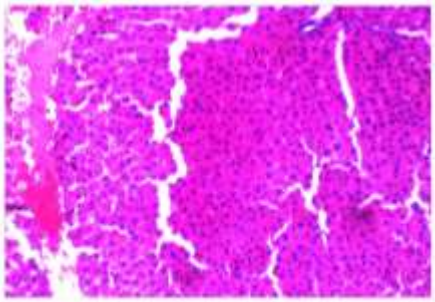
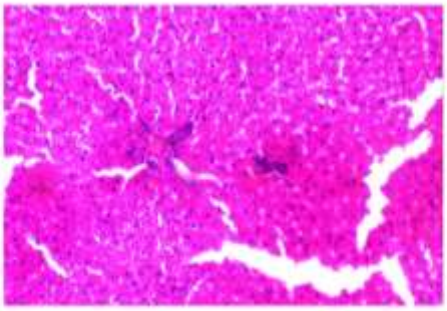


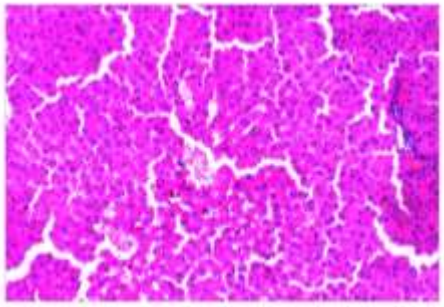
Group I - Normal



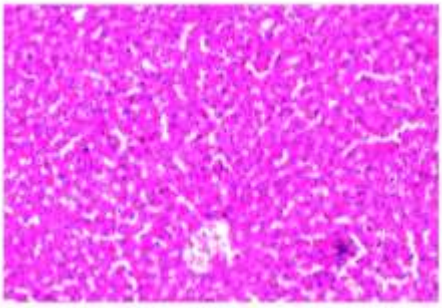
Group II - 1,4 DCB treated



Group III - Silymarin (100mg / kg / b.wt)



Group IV - *A. Neriifolia* (100mg / kg / b.wt)



Group V - *A. Neriifolia* (300mg / kg / b.wt)

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