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PROTECTIVE ROLE OF METHANOLIC FLOWER EXTRACT OF *ALLAMANDA NERIIFOLIA* HOOK AGAINST 1, 4 DICHLOROBENZENE (DCB)-INDUCED HEPATOTOXICITY

Sumathi R*and Anuradha R

Assistant Professor, Department of Biochemistry, Rabiammal Ahamed Maideen College for Women, Thiruvarur.

Abstract:

The present study was conducted to evaluate the protective role of methanolic flower extract of Allamanda neriifolia on 1, 4 dichlorobenzene (DCB)-induced hepatotoxicity in rat model. 1,4 DCB administered rats (300mg/kg/b.wt which was dissolved in 1ml of corn oil by intraperitonial injection for 45 days) were pre treated with methanolic flower extract of Allamanda neriifolia (300 mg / kg body weight) for 45 days and sacrificed after 1,4 DCB intoxication. Results showed that 1,4 DCB caused a marked rise in serum alanine aminotransferase (ALT), total cholesterol (TC), as well as marked decrease in serum total protein (TP), albumin (ALB), PCV, WBC, RBC, Hemoglobin and platelet compared to controls. However pre treatment with A.neriifolia methanolic flower extract produced a significant decrease in the ALT and total cholesterol level and an increase in total protein, albumin and hematological parameters compared to DCB alone group. Taken these data together, it can be concluded that natural plant components such as A.neriifolia flowers could protect the liver against dichlorobenzene (DCB)-induced liver toxicity.

Keywords: *Allamanda neriifolia, 1, 4 Dichlorobenzene, methanolic extract, Hepatotoxicity*

Corresponding author:

Sumathi R,

Assistant Professor,
Department of Biochemistry,
Rabiammal Ahamed maideen College for Women,
Thiruvarur.

E-mail: *sumathiraguraman@ymail.com*

Mobile no: 7667202905

QR code

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INTRODUCTION:

Liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. About 20,000 deaths occur every year due to liver disorders. Liver diseases are mainly caused by toxic chemicals such as certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride, chlorinated hydrocarbons and excess consumption of alcohol, infections and autoimmune disorders [1]. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages [2]. 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. A probable mechanism for the carcinogenic effects of mothballs and some types of air fresheners containing 1, 4 -DCB has been identified [3].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 [4]. Many drugs are known to cause hepatic injury. Conventional and synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Steroids, vaccines, and antiviral drugs, have been used as therapies for liver pathologies and have potential adverse side-effects, especially if administered chronically or sub-chronically. Current medical treatments for these liver diseases are often ineffective and therefore efforts are being made to seek new effective medications [5].

Allamanda neriifolia Hook is a genus of flowering plants in the dogbane family, Apocynaceae. They are native to the Americas, where they are distributed from Mexico to Argentina. Some species are familiar as ornamental plants cultivated for their large, colourful flowers. It has been used as a purgative or emetic, febrifuge as well as for the treatment of coughs, headaches, jaundice and enlarged spleen resulting from malaria. The milky sap is also known to posses antibacterial and possibly anticancer properties [6]. Preliminary phytochemical studies and GC-MS analysis were already done by using this flower. The presence of flavonoids in flowers of different *Allamanda* species and quantify the rutin by high performance liquid chromatography were evaluated [7]. Several flavonoids in the flowers extract were detected including rutin and the *Allamanda* is an excellent font of flavonoids.

Therefore, in this study, the ability of activity directed extracts of flowers of *Allamanda neriifolia* to protect the liver cells against 1, 4 DCB-induced hepatocellular damage in rats *in vivo* is investigated.

MATERIALS AND METHODS

Collection of plant

The flowers of *Allamanda neriifolia* Hook was collected from Tiruchirappalli and it was authentified by Dr.S.JohnBritto, Director, RAPINAT Herbarium and Centre for Molecular Systematics, St.Joseph'sCollege,Tiruchirappalli (Voucher No: 002) dust were removed from flowers and was dried at room temperature. These dried materials were macerated to powder and stored in air tight container for further use.

Preparation of extracts

300gm of coarsely ground powder was packed into soxhlet column and extracted with 250ml of 70% methanol for 48 hours (64.5-65.5°C). The extract was filtered and concentrated on water bath at reduced pressure (bath tem 50°C) to syrup consistency (yield: 15%). Then the dried extract was stored in air tight container for further use.

Experimental 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±2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water was provided *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).

Source of Drugs and chemicals

All the chemicals and solvents were of analytical grade and were purchased from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

Wistar albino rats were randomly divided in to five groups of 6 animals each.

- **Group I**-Negative control rats. Diet and water were available *ad libitum*
- **Group II**-Rats were administered with 1, 4 Dichloro benzene alone (300mg/kg/b.wt) which was dissolved in 1ml of corn oil by intraperitonial injection for 45 days.

- **Group III**-1, 4 Dichloro benzene induced rats received orally (standard drug) silymarin (100 mg/kg/b.wt) for 45 days.
- **Group IV**-1, 4 Dichloro benzene induced rats received with methanolic flower extracts of *A.Neriifolia* orally at a dose of (300 mg/kg/b.wt) for 45 days.
- **Group V**- Rats received with methanolic flower extract of *A.Neriifolia* alone orally at a dose of (300 mg/kg/b.wt) for 45 days.

After 45 days of treatment, the animals were fasted for 12 hrs and sacrificed by cervical dislocation. Blood was collected in a dry test tube and allowed to coagulate at ambient temperature for 40 min. Serum was separated by centrifugation at 2000 rpm for 10 minutes.

Biochemical analysis

Serum AST [8], Serum total protein and albumin [9] and Serum total cholesterol [10] were estimated. Haematological parameters like RBC [11], WBC [12], Haemoglobin [13], and platelet count [14] and PCV were also determined [15].

Statistical analysis

All quantitative measurements were expressed as mean ± S.E. for control and experimental animals. Data's were analysed by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using statistics software package for social sciences, personal computer (SPPS/PC).The results were considered statistically significant if the p value is less than 0.05.

RESULTS:

Administration of single dose of 300 mg/kg body weight of 1, 4 DCB to wistar rats produced significant changes in the biochemical parameters when compared to the control group. ALT and total cholesterol were significantly elevated ($P < 0.05$), while the serum total protein, and albumin levels were reduced in 1, 4 DCB -treated rats as compared to the control group (Tables 1). However pre-treatment of the rats with 300mg/kg of methanolic extract of *Allamanda neriifolia* flower produced a significant reduction in the levels of ALT and total cholesterol as well as significant increase in the levels of total protein, globulin and albumin compared to values in the DCB alone treated group. 1, 4 DCB treatments also significantly decreased WBCs, platelet, haemoglobin, RBCs, and PCV in DCB alone treated group compared to other groups (Table 2). However, pre-treatment with *Allamanda neriifolia* significantly mitigated the induced changes in hematological parameters.

Table 1: Effect of *A.neriifolia* and 1, 4 Dichlorobenzene on ALT, Total cholesterol, Total protein and Albumin in control and experimental rats

Groups	ALT(IU/L)	Total cholesterol (mg/dl)	Total protein(g/dl)	Albumin(g/dl)
Group I	38.9 ± 1.05^{a}	86.41 ± 2.14^{a}	8.9 ± 0.13^{d}	3.49 ± 0.85^{d}
Group II	81.3 ± 2.57^{d}	126.4 ± 3.42^{b}	5.50 ± 0.17^{a}	1.90 ± 0.22^{a}
Group III	50.1 ± 1.53^{b}	84.15 ± 2.85^{a}	7.56 ± 0.20^{c}	2.25 ± 0.49^{b}
Group IV	68.9 ± 1.70^{c}	90.12 ± 3.25^{a}	6.80 ± 0.24^{b}	2.17 ± 0.54^{b}
Group V	48.3 ± 1.30^{b}	88.32 ± 3.38^{a}	8.40 ± 0.29^{d}	3.17 ± 0.85^{c}

Note: Values are mean ± SE for six rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (Duncan's multiple range test).

Table 2: Effect of *A.neriifolia* and 1, 4 Dichlorobenzene on RBC, WBC, Hb, PCV and platelets in control and experimental rats

parameter	Group-I	Group-II	Group-III	Group-IV	Group-V
RBC (millions/cu.mm)	6.55±0.16^{c}	3.12±0.94^{a}	5.11±0.14^{b}	4.92±0.15^{b}	6.83±0.12^{c}
WBC (cells/mm^3)	8296±281.1^{b}	6118±182.4^{a}	8073±255.3^{b}	8319±180.4^{b}	8613±252.9^{b}
Hb (g/dl)	12.7±0.51^{c}	7.3±0.20^{a}	11.35±0.34^{b}	12.36±0.32^{b}	13.02±0.43^{c}
Platelets (10^5 cells/cu.mm)	287.66±11.17^{d}	134.0±4.11^{a}	191.3±4.14^{b}	219.66±5.45^{c}	274.3±9.91^{d}
PCV (%)	40.36±0.72^{b}	31.66±0.78^{a}	42.85±1.35^{c}	39.40±1.42^{b}	48.4±0.65 ^{d}

Note: Values are mean ± SE for six rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (Duncan's multiple range test).

DISCUSSION:

Liver disease and toxicity is common, especially with many drug treatments. Serum activities of AST and ALT are the most commonly used biochemical markers of liver injuries. Level of ALT, a marker enzyme of liver injury increased ($p<0.05$) significantly in 1, 4 DCB treated group when compared to normal control and extract pre-treated groups (Table1).The increase in activities of ALT, a liver marker enzyme in the serum of DCB induced rats indicates damage to hepatic cells. The abnormal high level of serum ALT in this study is a consequence of DCB-induced liver dysfunction. ALT is a better parameter for detecting liver injury and is more specific to the liver. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [16]. The increase in serum level of AST and ALT has been attributed to the damaged structural integrity of the liver. This is because they are cytoplasmic in their location and are released into circulation after cellular damage [17] .The result of this study however demonstrated that pre treatment with methanolic flower extract of *A.neriifolia* significantly caused a decrease in serum ALT in comparison to DCB induced group. Thus the extract protected the hepatocytes from toxicity.

Hepatotoxic substances are known to cause impairment of cholesterol metabolism leading to an increase in serum levels of cholesterol causing fatty liver [18]. Lipids are precursors for hormones, and are used for energy storage, and have a prominent role as messengers and regulators of inflammation [19]. Moreover, lipids are one of the most susceptible targets of free radicals [20].This oxidative stress induced lipid peroxidation causes many pathological events. The results of the present study have established that, the 1, 4 DCB treatment could have affected the lipid metabolism of liver cholesterol levels. It can be noted that hypercholesterolemia in DCB intoxicated rats was resulted from damage of hepatic parenchymal cells that lead to disturbance of lipid metabolism in liver [21]. However, rats are treated with *Allamanda neriifolia* extract showed a significant ($p < 0.05$) decline in cholesterol values compared to DCB-intoxicated alone rats.

In our study injection of 1, 4 DCB caused a significant reduction in the serum levels of total protein and albumin, further indicator of liver toxicity [22].Decrease in total protein and albumin may be associated with the decrease in the number of hepatocytes which in turn may result into the decreased hepatic capacity to synthesize protein.

Deleterious effect of foreign compounds, toxins, chemicals and plant extract on blood constituents of humans and animals can easily be detected through assessment of haematological parameters [23].Certain drugs including alkylating cytotoxic agents also affected blood formation rate and hematological parameters [24]. In the present study, a significant reduction of hematological parameters (RBC, WBC, Hb, Platelet count and PCV levels) in DCB induced group as compared with control group, was observed.

Administration of 1, 4 DCB to rats increases the erythrocyte membrane peroxidation, which may also lead to haemolytic changes. Hb concentration

decreased due to reduction in the oxygen-carrying capacity of blood and less amount of oxygen delivered to the tissues. Due to less supply of oxygen carrying capacity of blood PCV value was also decreased in 1, 4 DCB treated group and it clearly indicated that an induction of anemia. The level of WBC in chemical control rats were significantly decreased than normal control and it indicates a decline in the production of leukocytes called leukopenia, means that the body is less able to fight of infections [25]. Studies has shown that decrease in WBC count could be as a result of bone marrow deficiency or failure due to infection, cancer treating drugs that damage the bone marrow, disease of the liver or spleen. It had been reported that a significant decrease in the WBCs of the blood indicates a decline in the production of the defensive mechanism to combat infections, a situation which would naturally make the animal more susceptible to various physiological stress resulting in diseases, greater mortality and poor growth [26].

The reduction of these hematological parameters may be attributed to the hyperactivity of bone marrow, which leads to the production of red blood cells with impaired integrity that are easily destroyed in the circulation, as well as marked leucopenia [27].Our study indicated that altered the hematological parameters in 1, 4 DCB treated experimental rats were significantly improved to control base line by treatment with the extract of *A.neriifolia* (300mg/kg). This study was strengthened in the study of *Clerodendron inerme (L*) extract [28].

CONCLUSION:

The present data records that 1, 4 DCB intoxication produces a perturbation in hematological indices associated with an increase in ALT and Total cholesterol and provoke also Total protein and albumin levels depletion. Accordingly, care must be taken into account to avoid mammalian and human exposure to 1, 4 Dichlorobenzene. The co-administration of methanolic extract of *allamanda neriifolia* attenuates the observed harmful effects on all the parameters cited above. On the basis of this study, it should be taken into consideration that the supplementation of natural antioxidants may act as a protective agent against the toxicity of chlorinated benzenes.

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