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Evaluation of hepatoprotective activity of ethanolic polyherbal extract

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ABSTRACT

The effect of the ethanolic polyherbal extract of the leaves of *Melia Azadirachta,* Seeds of *Piper longum* and leaves of *Eclipta alba* belonging to the different family were studied for hepatoprotective activity against albino rats with liver damage, induced by Cadmium Chloride ($CdCl_2$) . Significant hepatoprotective effects were obtained in liver damage induced by Cadmium Chloride as evident from decreased enzyme levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), serum bilirubin (SB) in the Ethanolic *Polyherbal extract* treated groups (100, 200 mg/kg), compared to the intoxicated controls. The hepatoprotective effect was also supported by histopathalogical studies of liver tissue. Thus the present study provides a scientific rationale for the traditional use of this plant in the management of liver disorders.

Key words: *Melia Azadirachta, Piper longum, Eclipta alba,* Polyherbal extract, Cadmium Chloride ($CdCl_2$), Hepatoprotective activity.

1. INTRODUCTION

Every plant on this earth is useful for human beings, animals and other plants. Liver is the key organ to regulate homeostasis in the body and involved in almost all the biochemical pathways [1]. The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate [2]. Many plant products have been reported to protect against hepatic injury [3]. Presently only a few hepatoprotective drugs and that too from natural sources are available for the treatment of liver disorders.*Melia Azadirachta* belongs to the family Meliaceae, used orally and topically as medicine traditionally in many countries as an antiviral agent, diuretic, febrifuge and used against intestinal disorders [4,5]. A number of flavonoids, limmonoids, tannins, sterols, saponins and triterpenoids have been reported in this plant [6,7,8,9,10,11]. Its hepatoprotective activity, regeneration of hepatocytes by inhibition of free radicals generation, has been reported by Samudram et al., 2008 [5].

Piper longum Linn. belongs to the family Piperaceae, is a common Indian dietary spice which has been shown to possess a wide range of therapeutic utilities. It contains various alkaloids like piperine, piperlongumine, piperlonguminine, etc. It has been reported to possess, antiasthamatic,antiinflammmatory, hepatoprotective, hypocholestremic and immunomodulatory activities [12].*Eclipta alba* (L.) Hassk, commonly known as bhringraj, is a plant belonging to the family Asteraceae. In ayurvedic medicine, the leaf extract is considered a powerful liver tonic. It contains mainly coumestans ie., Wedelolactone (I), demethylwedelolactone (II) and Polypeptides, Polyacetylenes, thiophene derivatives, steroids, triterpenes and flavanoids. Wedelolactone possesses a wide range of biological activities and is used for the treatment of hepatitis and cirrhosis [13]. Apart from the traditional uses, these plants in the treatment of liver diseases, no systematic scientific study has been undertaken to evaluate the hepatoprotective activity. Hence attempt was made to assess the hepatoprotective activity of polyherbal extract of these plants against $CdCl_2$ induced hepatotoxicity in rats.

2. MATERIALS AND METHODS

2.1. Collection of the plant materials

The plant materials used for the polyherbal formulation (PHF) preparation were Melia *Azadirachta, Piper longum* Linn, *Eclipta alba* (L.) The plants were collected from Thiruninravur, Thiruvallur district, Tamilnadu, India. They were identified and authenticated by Prof.P.Jayaraman, Ph.D, Director, National Institute Of Herbal Science Retd, Professor, Presidency College, Chennai, Tamilnadu, India. Voucher specimens were deposited at the herbarium collection of the department of Pharmacognosy.

2.2. Extraction and preparation of polyherbal formulation

The leaves of *Melia Azadirachta,* seeds of *Piper longum* and whole plant of *Eclipta alba* were shade-dried and made into coarse powder. 100 gm of each plant powder were extracted by Soxhletion method with ethanol. Then the solvent is filtered and evaporated to dryness.

2.3. Preliminary phytochemical screening

The preliminary qualitative phytochemical studies for ethanolic polyherbal extracts of *Melia Azadirachta, Piper longum* and *Eclipta alba* were performed to screen the presence of different chemical groups such as alkaloids, tannins, glycosides and saponins etc [14, 15].

2.4. Experimental Animals

Male Wistar albino rats (150-200 g) were obtained from the animal house of Central Animal House of Darshan Institute of Pharmacological Studies, Puliyur, Karur TK, Tamilnadu. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 h ad libitum. All animal experiment was carried out in accordance with the guidelines of CPCSEA and study was approved by the IAEC (Institutional animal ethical committee).

2.5. Acute oral toxicity studies

Acute oral toxicity was performed by using OECD guidelines – 423 (Organisation of Economic Co-Operation Development) – Fixed Dose Procedure. The purpose of this study is to allow selection of the appropriate starting dose for the main study.Acute oral toxicity of was performed in Wistar Albino Rats. The rats were kept for 4 hr of fasting prior to the experiment and body weight of the rats should be noted. Usually rats weighting 150 – 200 gm were used for acute toxicity studies. The dose was given to every rat orally according to body weight. The test for acute toxicity was performed at 5, 50, 300, and 2000mg/kg oral dose of Ethanolic Polyherbal extract. Food was given for a 1-2 hours after the administration of drug.

During the first 4 hr, after the drug administration, animals were continuously observed for gross behavioral changes & then observation is continued for 24 hr & 72 hr in regular intervals for 14 days. The parameter such as hyperactivity, grooming, convulsions, sedation, hypothermia and change in fur colour, mortality, moribund stage or death were observed [16].

To induce acute liver injury, Cd ($CdCl_2$, 3 mg/kg body weight) will be dissolved in normal saline and intravenously (i.v.) injected into the rats. This dose is selected because it severely elevates plasma alanine aminotransferase (ALT) levels in a previous experiment that evaluated the Hepatoprotective activity of $GdCl_3$ [17].

2.6. Experimental design for Hepatoprotective activity

Five groups containing six animals each were used for the study. The animals from Group I served as the control and received the vehicle for 3 days. Groups II–V received 3mg-/kg/ day i.p. of $Cdcl_2$ for1 day (24 h after the last dose of polyherbal extract). The standard drug Liv- 52 syrup (2ml/100g) p.o, once daily for 3 consecutive days (Himalaya drug Company, India) [18] was administered to Group V animals. While, Groups III and IV (100mg/kg, 200mg/kg) were treated with Ethanolic polyherbal extract for 3 days, respectively.All the animals were killed on day 5 under chloroform anaesthesia. The blood samples were collected separately by Retero orbital punctures into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 min and was used for the estimation of some biochemical parameters like Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), Serum bilirubin (SB) by using automated blood analyser.

2.7. Histopathology

After draining the blood, liver samples were excised, washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Paraffin sections were taken at 5 mm thickness, processed in alcohol–xylene series and were stained with alum hematoxylin and eosin. The sections were examined microscopically for histopathological changes [19].

2.8. Statistical analysis

Values are expressed in mean±S.E.M. for six animals in each group. P-value was calculated using ANOVA followed by Dunnett's test for multiple comparisons. Values of $P < 0.001$ were considered significant in all cases.

3. RESULTS AND DISCUSSION

3.1. Preliminary Phytochemical Screening

Preliminary pytochemical screening revealed the presence of Sterols, Proteins,

Terpenoids, Fixed oil, Alkaloids, Flavonoids, Carbohydrates Gums and mucilage.

3.1. Acute toxicity studies

Acute toxicity studies showed no mortality up to the doses of 2000 mg/kg body weight. So, the extract is safe for long term administration.

3.3. Cadmium Chloride Induced Hepatotoxicity

Liver, contains large amount of marker enzymes [20], is an important metabolic organ involved in the synthesis of large number of metabolites. The results of biochemical parameters have shown the elevation of enzyme level in $CdCl_2$ treated group, indicating that $CdCl_2$ induces damage to the liver (Table 1). Hepatocellular necrosis leads to very high level of AST and ALT in blood released from liver to blood.

A significant reduction ($P< 0.001$) was observed in ALT, AST, ALP and Serum bilirubin levels in the groups treated with Liv. 52 and ethanolic polyherbal extracts, indicating the regeneration of damaged liver cells by the standard and test drug. Alanine transaminase is a better index of injury, as its activity represents 90% of total enzymes present in the body. The decrease in serum transaminase concentration indicates the stabilization of plasma membrane and protection of hepatocytes [21]. The hepatoprotective effect of ethanolic polyherbal extract may be due to its flavonoid content[22].

Histopathological examination of the liver treated with $CdCl_2$ showed disarrangement of normal hepatic cells with centrilobular necrosis and vacuolization. The control group showed normal cellular architecture with distinct hepatic cells and sinusoidal spaces. The liver sections of the rat treated with 100, 200 mg/kg bodyweight p.o of ethanolic polyherbal extract followed by Cadmium chloride intoxication showed less vacuole formation and absence of necrosis and overall less intense in centrilobular necrosis and vaculization were observed with standard Liv. 52, supplementing the protective effect of the test drug and the standard hepatoprotective drug.

Effect of Ethanolic polyherbal extract (EPE) on $Cdcl_2$ induced liver damage in rats.

(Fig. 1) vehicle treated - showing normal architecture of hepatic cells (H & E, x 150);

(Fig. 2) $Cdcl_2$ treated - showing inflammatory infiltration and severe fatty changes;

(Fig. 3) liver pretreated with EPE (100mg/kg) prior to $Cdcl_2$ administration - showing a pattern of moderate fatty change;

(Fig. 4) liver pretreated with EPE (200mg/kg) prior to $Cdcl_2$ administration - showing mild fatty change (H & E, x 450);

(Fig. 5) liver pretreated with Liv 52 prior to $Cdcl_2$ administration – showing normal architecture with less fatty changes.

Figure-1: Control

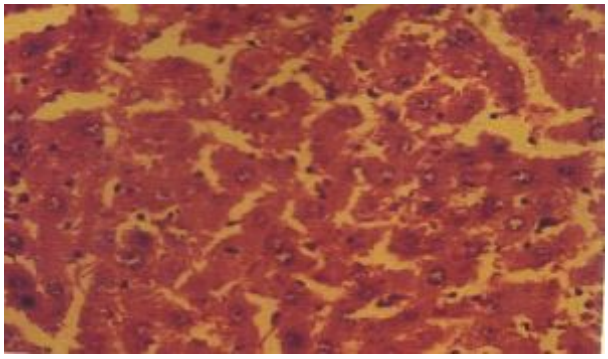

Figure-2: $CdCl_2$

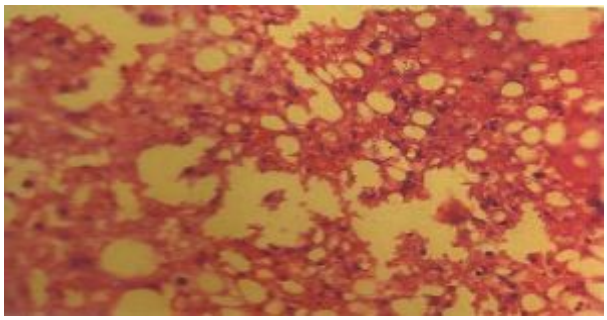

Figure-3: EPE 100 mg/ng + $CdCl_2$

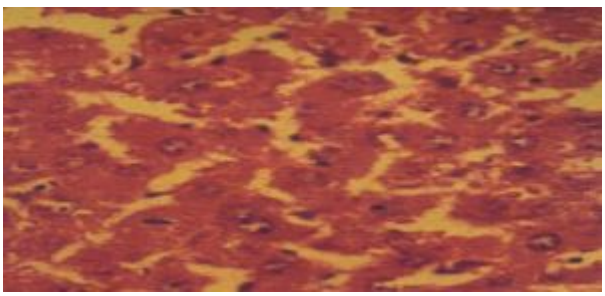

Figure-1: EPE 200 mg /Kg + $CdCl_2$

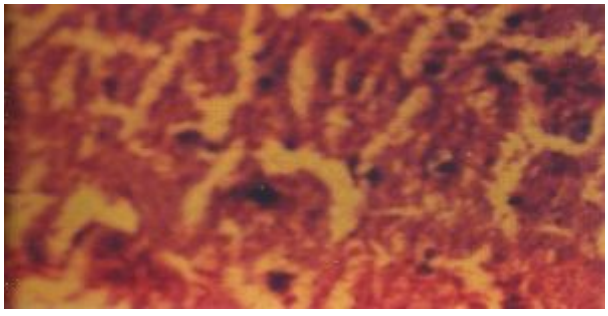

Figure-2: Liv. 52 + $CdCl_2$

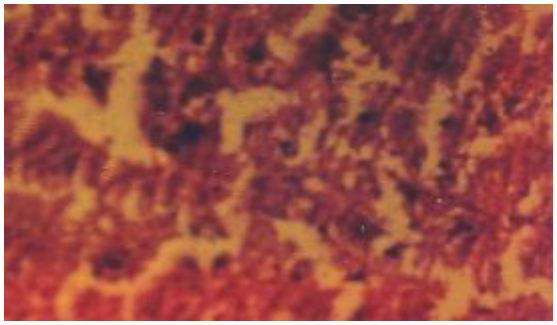

Table-1: Effect of Ethanolic polyherbal extract *on* serum enzyme and biochemical parameters in $CdCl_2$ induced hepatic damage in rats

GROUP	DOSE	ALT	AST	ALP	SB
Control	5 ml	54.83 ± 0.60	164 ± 0.96	165.16 ± 1.13	1.01 ± 0.003
$CdCl_2$ (Control)	0.3 ml	306 ± 1.29 [a]	376.16 ± 0.60 [a]	486 ± 1.39 [a]	2.23 ± 0.01[a]
EPE + $Cdcl_2$	100 mg / kg	144. 16 ± 1.16 [a]	243 ± 0.80 [a]	284.33 ± 0.76 [a]	1.26 ± 0.06[a]
EPE + $Cdcl_2$	200 mg / kg	126.5 ± 0.76 [a]	215.16 ± 1.13 [a]	225.83 ± 1.30 [a]	1.15 ± 0.01[a]
Liv 52 + $Cdcl_2$	25 mg / kg	53 ± 1.39 [a]	149.5 ± 2.24 [a]	152.16 ± 4.41 [a]	0.9 ± 0.01 [a]

4. CONCLUSION

Preliminary phytochemical studies in our laboratory have indicated the presence of flavonoids in *Poly herbal extract* it is likely that the flavonoids of EPE, may be responsible for the hepatoprotective activities of Extract. Flavonoids, consumed in large amounts in the daily diet, are helpful to protect the liver. The results of the present study demonstrate that the polyherbals posses' potent hepatoprotective action on Cadmium Chloride induced hepatic damage in rats. Further, phytochemical studies are in progress to isolate, characterize and identify the specific active flavonoid in this polyherbals responsible for liver protection.

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