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Research Article

Hepatoprotective Activity of *Ervatamia Heyneana* Leaves Extracts on Carbon Tetrachloride Induced Hepatotoxicity in Rats

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ABSTRACT

The present study was aimed at investigating hepatoprotective activity of different extracts of leaves of *Ervatamia heyneana* with a view to justify the use of the plant. The various extracts of leaves of *Ervatamia heyneana* were obtained by successive soxhlation with petroleum ether (60 - 80 ° C), chloroform, ethyl acetate, methanol and water for 6 hours. The dried extracts of leaves (Petroleum ether, methanol and aqueous) were used for hepatoprotective activity against carbon tetra chloride induced liver damage in albino rats. Various biochemical parameters were studied to evaluate the hepatoprotective activity of the extracts for total bilirubin (TBL), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein (TPL) and albumin levels (ALB). This study revealed that aqueous extract (500mg/kg) showed significant activity which was comparable to that of standard drug silymarin (100mg/kg). Aqueous extract (500mg/kg) was found to protect the rats from hepatotoxic action of carbon tetra chloride as evidenced by significant reduction in the elevated serum enzyme levels. Histopathological studies showed regeneration of hepatocytes of the extract treated liver samples which stipulate its hepatoprotective activity.

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INTRODUCTION

Liver is the principal and most core intricate organ of the body. It plays a major role in detoxification and excretion of many endogenous and exogenous compounds. Any impairment or injury of the liver may result in many consequences on one's health. The frequent target for various toxicants is liver which is actively involved in many metabolic tasks [1]. Hepatic damage is linked with falsification of these metabolic activities [2]. In experimental studies, Carbon tetra chloride is used widely to induce liver damage and toxicity of it has been extensively studied. Hepatic injury may result in leakage of cellular enzymes into the blood stream and centrilobular necrosis [3-5]. The extent of functional damage of liver can be analyzed by Liver function tests (LFTs). The progress or regress of the disease can be studied by repetitive LFTs. LFTs are helpful in identifying the cause of hepatic disordees [6]. *E. heyneana* is used in Ayurveda and Unani systems of medicine to treat various diseases. It has been reported to have antitumor and cytotoxic [7-10], antifertility [11,12] anti-implantation [13], anticholinergic and antihistaminic [14], hypoglycaemic activities [15]. Literature reviews revealed, use of *Ervatamia coronaria* as hepatoprotective against CCl4 induced hepatic damage [16]. However, hepatoprotective activity of *Ervatamia heyneana* has not been clinically evaluated so far. As well as, based on its traditional use as a tonic to the liver [17], the present study was aimed at evaluating the hepatoprotective activity of various extracts of leaves of *Ervatamia heyneana* against carbon tetrachloride induced hepatotoxicity in albino rats.

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MATERIALS AND METHODS

Collection of plant material

The leaves of *Ervatamia heyneana* were collected from Balasamudram, Warangal. After identification by a taxonomist, a voucher specimen was deposited in laboratory herbarium. The collected plant material was thoroughly checked for any foreign matter. Leaves were separated, shade dried, powdered with laboratory mixer and sieved.

Materials

Silymarin was obtained from Micro Labs, Bangalore. SGOT and SGPT kits were obtained from Crest biosystems, Goa. Kits for determination of alkaline phosphatase, albumin and total protein were obtained from M/s Excel Diagnostics Pvt. Ltd, Hyderabad. Bilirubin was obtained from Anamol Laboratories Pvt. Ltd. Palghar, Maharashtra. All the solvents were procured from E. Merck, Mumbai.

Procurement and maintenance of animals

Adult albino rats of either sex belonging to wistar strain weighing 150 – 200g were purchased from Mahaveera Enterprises, Hyderabad. The animals were acclimatized for about 7 days prior to dosing. Cage numbers and individual markings were made with a marker pen to identify the animals. The animals were maintained in well ventilated room temperature with natural 12 ± 1h day–night cycle in the propylene cages with a bedding of paddy. Pellet chow feed standard diet under good management conditions and water *ad libitum* was provided to the animals. The study was carried out in accordance to the guidelines prescribed by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Preparation of extracts

For the present investigation, the extracts of leaf powder of *Ervatamia heyneana* were prepared by successive soxhlation with petroleum ether (60 – 80 ºC), chloroform, ethyl acetate, methanol and water for 8 hours. Filtrate was concentrated and dried in desiccator. Weighed amount of dried extract was dissolved arachis oil [18] and subjected to hepatoprotective activity [19].

Pharmacological screening

For the present investigation, the animals were pretreated with test extracts before inducing liver damage with CCl_4. Seven days after acclimatization the rats were divided into nine groups (I- IX), each group consisting of six animals. All animals were kept on same diet for 7 days. Group I served as normal and received 1 ml/kg of arachis oil p.o. for seven days. Group II served as toxic control and was given 5 ml/kg of 50% v/v CCl_4 in olive oil intraperitoneally on the seventh day. Group III (standard) animals were administered with 100 mg/kg of silymarin p.o. for seven days, followed by CCl_4 administration i.p. on the seventh day. Group IV –IX were treated in a similar way to that of group III (standard) using petroleum ether, methanolic and aqueous extracts of *Ervatamia heyneana* leaves at doses of 250 and 500 mg/kg in place of standard respectively.

All the rats were anaesthetized with thiopentone sodium (60 mg/kg i.p.) 36h after administration of CCl_4. Blood was collected from common carotid artery by carefully opening the neck region of the rat. After collection, the blood samples were allowed to coagulate at room temperature for at least one hour. Serum was separated by centrifugation at 3000 rpm for 30 minutes and then analysed for total bilirubin (TBL) [20], alanine transaminase (ALT), aspartate transaminase (AST) [21], alkaline phosphatase (ALP), total protein (TPL) and albumin levels (ALB) [22]. Any toxicity to the liver can result in increase in ALT, AST and ALP and a decrease in TBL, TPL and ALB levels. The animals were dissected, the livers were carefully taken out and washed with normal saline solution and preserved in formalin solution (10% formaldehyde) for histopathological studies.

Histopathological examination of liver

The livers were excised from the experimental animals of each group after collecting the blood sample and washed with normal saline. Weight of liver of each animal was noted. After this, the livers were fixed in 10% buffered neutral formalin and with bovine solution they were processed for paraffin embedding following the standard microtechnique. The sections were processed in alcohol xylene series and were stained with alum haematoxylin and eosin. The sections were examined by a light microscope (Model No: 138, Linco Pvt Ltd, Ambala-cantt, Punjab, India) with 100X magnification for the evaluation of histopathological changes [21, 23].

Statistical analysis:

The data are presented as mean±SD and analyzed by one- way ANOVA, followed by Dunnett't' test. The results of all the extracts including the standard drug are compared with the result produced by control and it is considered as significant as $P < 0.05$.

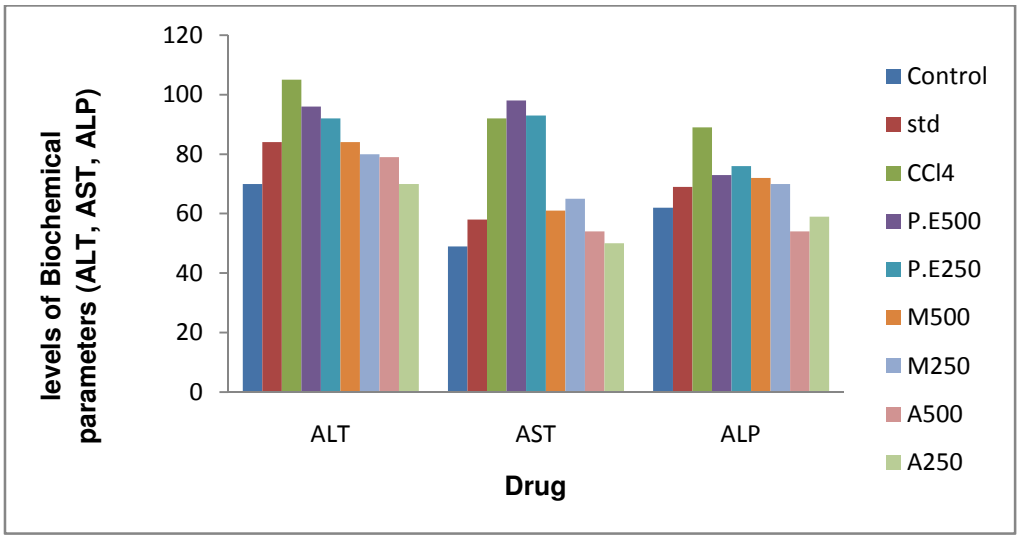
RESULTS

From the investigations (Table 1) it can be observed that the levels of ALT, AST, ALP, TBL reduced upon administration of the petroleum ether, methanol and aqueous extracts. TPL and ALB levels were found to increase with all the extracts. Among all the extracts, aqueous extract (500mg/kg) showed significant activity which

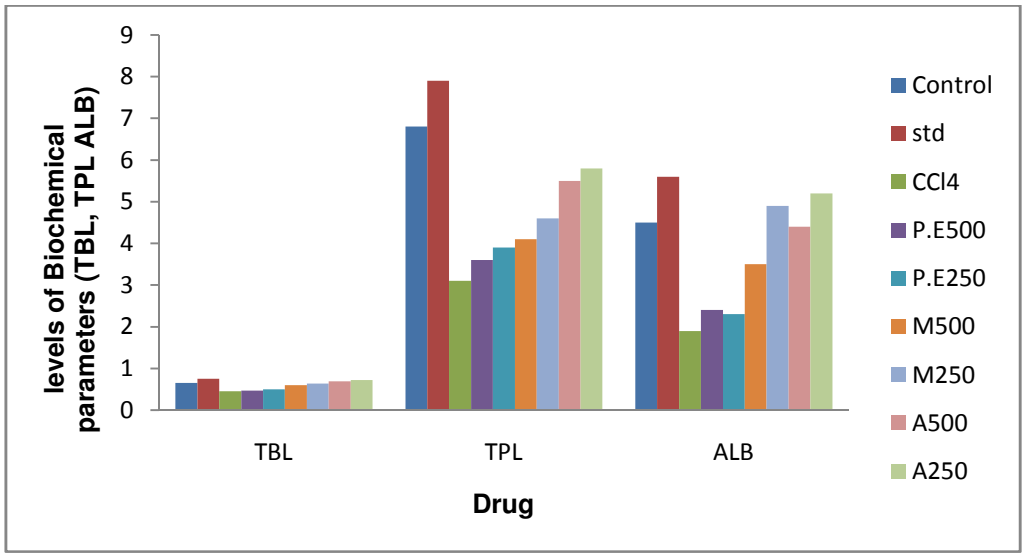
Table 1: Effect of Extracts on Different Serum Biochemical Parameters in CCl4 Induced Liver Toxicity

Groups	Dose (mg/kg)	ALT (U/ml)	AST (U/ml)	ALP(KA units/ml)	TBL (mg/dl)	TPL (mg/dl)	ALB (gm/dl)
Control	-	70±2.98	49±2.21	62±3.65	0.65±0.04	6.8±0.36	4.5±0.4
Standard	100	84±2.98	58±1.82	69±1.5	0.55±0.02	7.9±0.40	5.6±0.29
CCl4	-	105±3.30	92±3.65	89±2.58	0.75±0.05	3.1±0.18	1.9±0.25
Pet ether	250	96±3.41	98±2.94	73±2.94	0.47±0.04	3.6±0.18	2.4±0.18
	500	92±1.91	93±3.65	76±3.65	0.50±0.04	3.9±0.25	2.3±0.29
Methanol	250	84±3.65	61±3.36	72±2.58	0.60±0.04	4.1±0.18	3.5±0.25
	500	80±2.38	65±3.91	70±2.94	0.64±0.02	4.6±0.18	4.9±0.22
Aqueous	250	79±2.58	54±3.30	54±3.40	0.69±0.01	5.5±0.22	4.4±0.18
	500	70±3.65	50±2.75	59±3.5	0.72±0.01	5.8±0.40	5.2±0.18

Values expressed as mean ± SD, P value < 0.05



Graph 1: Effect of leaf extracts of Ervatamia heyneana on levels of various biochemical parameters (ALT, AST, ALP) in CCl4 induced liver toxicity



Graph 2: Effect of leaf extracts of Ervatamia heyneana on levels of various biochemical parameters (TBL, TPL, ALB) in CCl4 induced liver toxicity

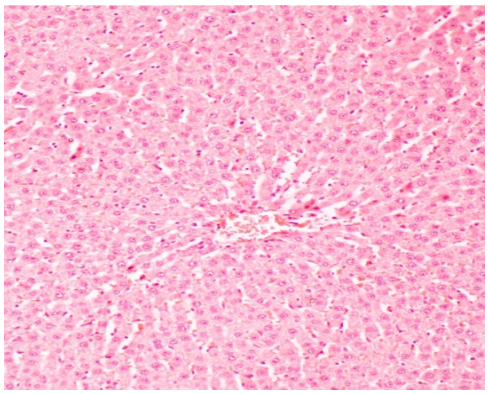


Figure 1: Liver of rat treated with Control

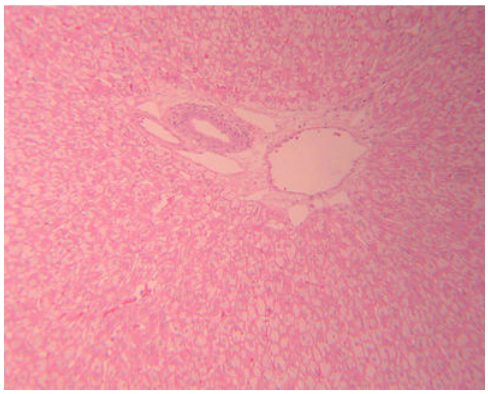


Figure 2: Liver of rat treated with petroleum ether extract 250 mg/kg

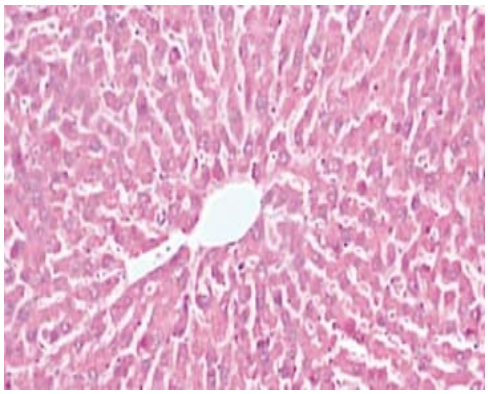


Figure 3: Liver of rat treated with petroleum ether extract 500 mg/kg

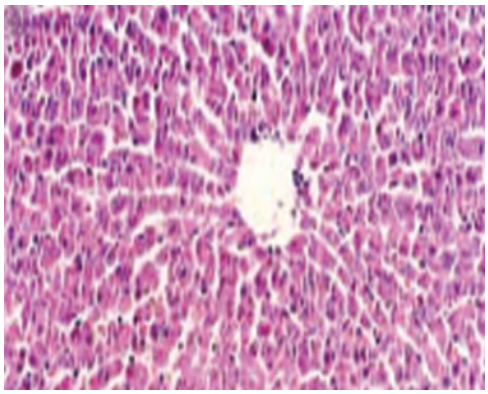


Figure 4: Liver of rat treated with methanol extract 250 mg/kg

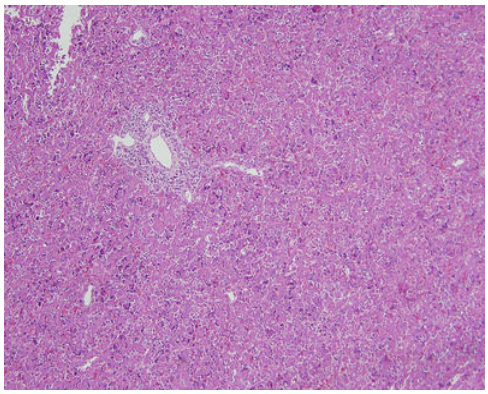


Figure 5: Liver of rat treated with methanol extract 500 mg/kg

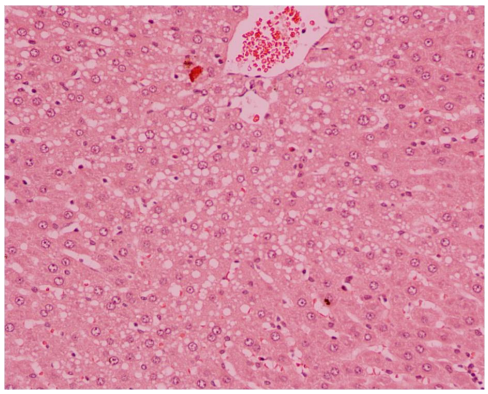


Figure 6: Liver of rat treated with aqueous extract 250 mg/kg

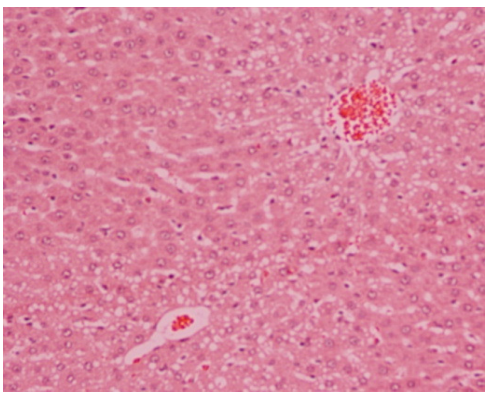


Figure 7: Liver of rat treated with aqueous extract 500 mg/kg

was comparable to that of standard drug silymarin (100mg/kg).The hepatoprotective activity of all the extracts was dose dependent with respect to ALT, AST, TBL and TPL assays. Histopathological examination of liver sections of control group showed normal cellular arrangement with distinct hepatic cells, sinusoidal spaces, and central vein. The liver sections of the group intoxicated with Carbon tetrachloride, vacuolization and irregular distribution of normal hepatic cells with necrosis was observed. The liver sections of the group treated with aqueous extract (500mg/kg) body weight p.o, followed by intoxication with carbon tetrachloride, showed less vacuole formation and absence of necrosis and less visible changes (Fig 7). This was comparable with standard Silymarin, projecting the hepatoprotective effect of the test drug. The activity of aqueous extract (500mg/kg) showed significant activity when compared with that of other extracts.

DISCUSSION

The present studies were performed to assess the hepatoprotective activity of various extracts of *Ervatamia heyneana* in rats, against hepatotoxicant, Carbon tetrachloride which is biotransformed to trichloromethyl free radical, by the cytochrome P-450 system. This free radical binds covalently to cell membranes and organelles and causes lipid peroxidation, disturb Ca 2+ haemostasis leading to cell death. In liver toxicity, changes in endoplasmic reticulum occur, metabolic enzyme activation is lost, protein synthesis is reduced and activation of glucose-6-phosphatase is lost, leading to liver damage. In our study, animals of Group II (toxic control) receiving Carbon tetrachloride significantly lost their body weight and showed reduced food consumption as compared to control group. Animals of Group III receiving Carbon tetrachloride plus standard drug Silymarin 100mg/kg body weight and Group IV - IX receiving Carbon tetrachloride plus 250 and 500mg/kg body weight of test extracts , showed a significant increase in body weight and food consumption when compared to Carbon tetrachloride treated group animals. These findings indicated that the toxic effects of Carbon tetrachloride were significantly neutralized by the extracts administered which were comparable to standard drug. Any toxicity to the liver can result in increase in ALT, AST, ALP and TBL and a decrease in TPL and ALB levels. Hence, the estimation of these biochemical parameters in the serum may be a useful to determine the extent of hepatocellular damage. The restoration of these enzyme levels to normal after administration of the extracts manifests clearly the antihepatotoxic effects of the extract. In the histopathological examination of liver sections in group IV - IX (test group) the normal architecture of the liver was retained when compared to group II (toxic control) and was almost comparable to that of group III (standard) and among all the extracts, hepatoprotective activity of aqueous extract 500 mg/kg body weight was comparable with the standard Silymarin group, thus, confirming the significant hepatoprotective effect of this extract.

CONCLUSION

In accordance with these results, it may be confirmed that aqueous extract 500 mg/kg body weight has significant hepatoprotective activity. For further progress of this study, specific active constituents from leaves of *Ervatamia heyneana* responsible for hepatoprotective effect can be identified.

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