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Research Article

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF HYDRO-ALCOHOLIC LEAF EXTRACT OF *ADHATHODA VASICA* ON CCl_4 INDUCED HEPATOTOXICITY IN SWISS ALBINO RATS

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A B S T R A C T

Hydro-alcoholic leaf extract of *Adhathoda vasica* is evaluated for hepatoprotective Activity in rats. The leaf extract (50 and 100 mg/kg, p.o.) showed a remarkable Hepatoprotective activity against carbon tetrachloride (CCl4) induced hepatotoxicity as judged from the serum marker enzymes and antioxidant levels. In hepatoprotective study, carbon Tetrachloride (CCl4) induced a significant rise in Aspartate amino transferase (AST), Alanine Amino transferase (ALT), Alkaline phosphatase (ALP), Total bilirubin, Total proteins, SuperOxide Dismutase (SOD) and decrease in Lipid peroxidation (LPO). 100 mg/kg p.o. of plantExtract has significantly ($P<0.001$) altered serum marker enzymes and antioxidant levels to Near to normal against carbon tetrachloride (CCl4) treated rats .The activity of the extract at Dose of 100 mg/kg was comparable to the standard drug, Silymarin (100 mg/kg, p.o) for Hepatoprotective activity. Histopathological changes of liver sample were compared with that of the respective control samples. The results indicate the hepatoprotective property of Hydro-alcoholic leaf extract of *Adhathoda vasica* exhibited a significant activity against Carbon tetrachloride induced hepatotoxicity in experimental rats.

INTRODUCTION

The liver plays a vital role in the metabolism of chemicals such as dietary uptake toxins in the body and such other substances in the circulatory system. The liver is expected to protect itself from the hazards of harmful drugs and Chemicals. There are many risk factors that cause liver diseases including hepatic virus, alcohol consumption and chemical agents have significant impact on the aetiology of liver diseases. The study of progress in pathogenesis of liver disease has been made to understand and to prevent the chronic liver disease. In spite of scientific advancements in the field of medicine there are still liver problems like jaundice Fibrosis and hepatitis which cause increase in mortality. The bile acids and toxicants like bilirubin are secreted concentrated and synthesized by the liver[1] Hepatotoxicity caused by drug induced injury to hepatocytes and bile duct cells and causes intrahepatic accumulation of toxic bile acids and excretion products which promotes for the hepatic injury.[2]Apoptotic cell death and necrotic cell death because of risk detection of drug induced injury the active proliferative response of hepatocytes make the liver an important target for carcinogens. The plant *Adhathoda vasica* belonging to the family of Acanthaceae is a small evergreen shrub that grows on the plains of India and in the lower Himalayans, up toa range of 1000 meters above sea level[3][4]The leaves and roots of *Adhathoda vasica* have anti-asthmatic and bronchodilator activity. The leaf powder of this plant containsanti-ulcer activity. A drug named bromo-hexine used in tuberculosis treatment is also obtained from this plant. This plant also contains anti-ulcerogenic activity, anti-allergyabortifacient activity and uterotonic activity, insecticidal activity and also anti-bacterial activity.[5]

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MATERIALS AND METHODS

Plant material

The leaves of *Adhathoda vasica* are obtained from the kodaicanal region of Tamil Nadu of India.

Preparation of Extraction

Fresh leaves of "*Adhatoda vasica*" are collected and shade dried. The leaf powder was macerated with hydro alcoholic solvent (30% v/v). Leaves are macerated for one week with occasional shaking at the interval of 5hrs. It was filtered with the help of filter paper and the extract was collected. This extract was heated on hot plate for complete evaporation of solvent. The extract was stored in refrigerator until use. Thus the preparation is used for further pharmacological evaluation.

Drugs and chemicals

Carbon tetra chloride, Silymarin were used for the study. All the solvents used for the extraction process are of Laboratory grade and they are purchased from local firms.

Animals

Six to seven weeks old female rats weighing about 150 - 200g were obtained from the animal house of having the REG.NO 006/IAEC/NCPA/B.PHARMACY/2018-2019. The animals were housed individually in stainless steel wire meshed plastic cages (in a temp 23±2) and humidity (55%-60%) controlled room with a 12h light-dark cycle. The animals were supplied with standard mice pellet diet and drinking water during the entire period of this study. Animals were maintained and experiment was carried out according to the rules and regulations of CPCSEA.

Experimental model

The animals were divided into 5 groups (n=6), Group-I (standard) treated with olive oil(vehicle), group -II (Negative control) treated with carbon tetrachloride(6mg/kg), Group - III (Standard group) treated with Silymarin (100 mg/kg)and Group -IV, V (test group) treated with extract (50 mg/kg and 100 mg/kg),. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC).

Biochemical parameters

The parameters like ALT, ALP, AST, Total bilirubin level, Total protein level and antioxidant parameters like SOD, LOP are estimated in the present experiment.

Biochemical Studies

Biochemical parameters were assayed according to the standard methods. Alanine aminotransferase (ALT), alkaline phosphate (ALP), aspartate aminotransferase (AST), total bilirubin level, total protein level and antioxidant parameters like SOD, LOP are estimated.

Estimation of Serum Aspartate Amino Transferase (AST) By Kinetic Method

The samples obtained from animals are labelled as blank, negative control, standard, test-1 and test-2 and incubate them at 37oC for 5 minutes. Take 100 µl of blank, negative control, standard, test-1 and test-2in separate tubes and add 0.8 ml of AST reagent to each tube. Cap the tube and mix well by inversion. Incubate reagent tubes at 37oC for 60 minutes. Add 0.5 ml of colour developer A to each reagent tube. Cap the tubes and mix well by inversion and the tubes were made to stand at room temperature for 20 minutes. Add 2 ml of colour developer B to each reagent tube. Cap the tubes and mix well by inversion and the tubes were made to stand at room temperature for 5 minutes. Place the blank tube in the test well and adjust the photometer to zero absorbance. Place the control and test tubes in the test well and record the absorbance of the control and test samples. [6]

$$\text{AST concentration value of control Absorbance of control sample} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of test sample}} \times \text{mean assay.}$$

Estimation of Serum Alanine Transaminase (ALT)

The samples obtained from animals are labelled as blank, negative control, standard, test-1 and test-2 and incubate them at 37oC for 5 minutes. Take 100 µl of blank, negative control, standard, test-1 and test-2in separate tubes and add 0.8 ml of ALT reagent to each tube. Cap the tube and mix well by inversion. Incubate reagent tubes at 37oC for 30 minutes. Add 0.5 ml of color developer A to each reagent tube. Cap the tubes and mix well by inversion and the tubes were made to stand at room temperature for 20 minutes. Add 2 ml of color developer B to each reagent tube. Cap the tubes and mix well by inversion and the tubes were made to stand at room temperature for 5 minutes. Place the blank tube in the test well and adjust the photometer to zero absorbance. Place the control and test tubes in the test well and record the absorbance of the control and test samples. [7].

$$\text{ALT concentration value of control Absorbance of control sample} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of test sample}} \times \text{mean assay}$$

Estimation of Alkaline Phosphatase (ALP)

To each tube 1 ml of reagent was added and is allowed to equilibrate at 37oC. The spectrophotometer was adjusted to zero with water at 405 nm and 0.025 ml (25 µl) of sample was added to reagent tube and was mixed well. After 1 minute, the absorbance was measured.Repeat readings every minute for next 2 minutes. Calculate the average absorbance difference per minute. The Abs/min multiplied by the factor 2187 will yield results in IU/L. Samples with values above 800 IU/L should be diluted 1:1 with saline, re assayed and the results multiplied by 2. [8]

$$\text{IU/L} = \frac{\text{Abs/min} \times 1000 \times \text{TV}}{\text{mM} \times \text{LP} \times \text{SV}} = \frac{\text{Abs/min} \times 1000 \times 1.025}{18.75 \times 1 \times 0.025}$$

Where as
Abs/min = Absorbance change per minute
1000 = Conversion of IU/ml to IU/L TV = Total volume (1.025 ml)
mM = Mllimolar absorptivity of p-nitro phenol (18.75)
LP = light path (1 cm)
SV = Sample volume (0.01 ml)

Estimation of Total Serum Proteins

Label test tubes blank, standard, control, test and add 1 ml of Biuret reagent to each tube. Add 0.02 ml (20 µl) of standard and test to appropriate tubes and mix by inversion and the tubes were made to stand at room temperature at 540 nm and zero absorbance with the reagent blank. Read and record absorbance readings of each tube [9].

$$\text{Total protein (g/dl)} = \frac{\text{Absorbance of unknown}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Estimation of Total Bilirubin

Label tubes as blank, standard, control, test. Each tube requires a blank tube. 1 ml of total Bilirubin reagent was added to all the blank tubes. The working reagent (Total Bilirubin working reagent: Add 0.05 ml (50 µl) of Sodium nitrate to 1 ml of total Bilirubin reagent and mix well) was prepared and 1 ml of this reagent was taken in labelled test tubes, except blank and 0.1 ml (100 µl) of each standard, control and sample to its respective tube and mix well. All the tubes were made to stand for 5 minutes at room temperature. Set the wavelength of the

instrument at 560 nm. Zero with reagent blank. Read and record absorbance of all tubes [10].

$$\text{Total Bilirubin (mg/dl)} = \frac{\text{Abs of unknown-Abs of blank}}{\text{Abs of calibrator-Abs of calibrator blank}} \times \text{Concentration}$$

Estimation of Lipid Peroxidation (LPO)

To 0.1 ml of the liver homogenate, 2.0 ml of 20% TCA was added. The contents were mixed well and centrifuged at 4000 rpm for 20 minutes. 2.0 ml of the supernatant was mixed with 2.0 ml of Thio-barbituric acid reagent. Reagent blank standard [11]

Histopathology

The liver was isolated from the dissected animals and is placed in 10% formalin solution and is made undergo a series of steps for the microscopic observation of histopathological changes in liver. This evaluated liver is scored according to the severity of the hepatic toxicity.

RESULTS AND DISCUSSIONS

Effect of Hydro Alcoholic Leaf Extract (HALAV) of "Adhatoda vasica" on Biochemical Parameters

The hydro alcoholic leaf extract of "*Adhatoda vasica*" is given at the dose of 50mg/Kg and 100mg/Kg and the biochemical parameters are estimated and the values obtained are almost equal to that of standard. The biochemical values are

Table 1 Effect of HALAV on biochemical parameters

Animals	ALT	AST	ALP	T.B	T.P
normal	52.85±0.142	78.34±0.783	112.58±0.142	0.87±0.07	7.17±0.14
Negative control	134.2±0.913*	178.15±0.858*	198.52±0.913*	2.85±0.02*	5.65±0.63*
Standard	53.29±0.479**	82.68±0.648**	115.8±0.479**	6.92±0.03**	6.92±0.14**
Test 1	89.17±0.512**	112.05±0.999**	127.41±0.512**	6.94±0.09**	6.94 ±0.87**
Test 2	54.81±0.219**	93.26±0.571**	114.43±0.219**	6.86±0.2**	6.86±0.12**

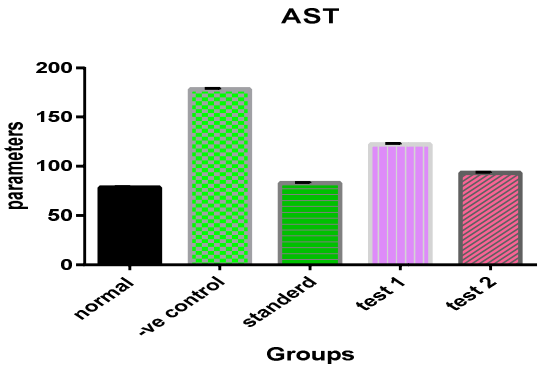

Figure 2 Effect of HALAV on AST

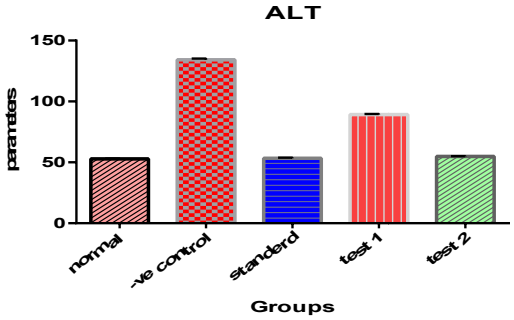

Figure 2 Effect of HALAV on ALT

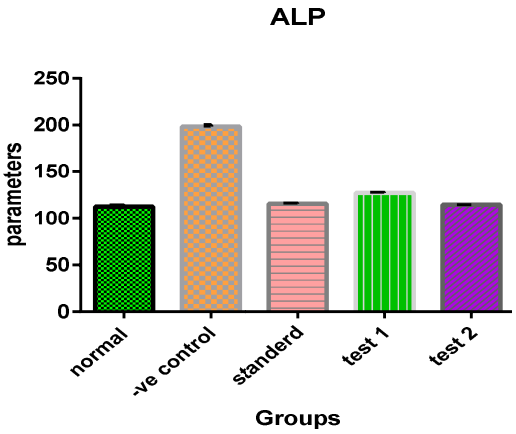

Figure 3 Effect of HALAV on ALP

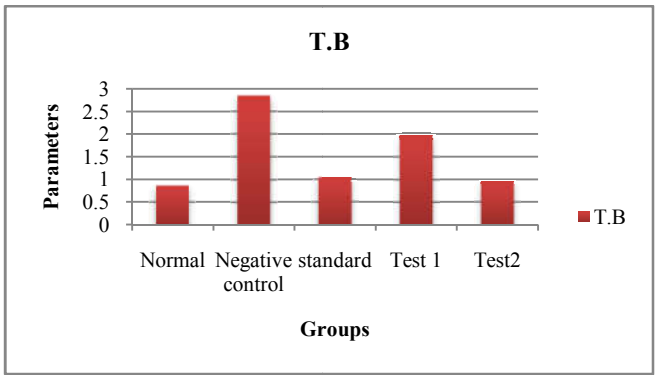

Figure 4 Effect of HALAV on ALT

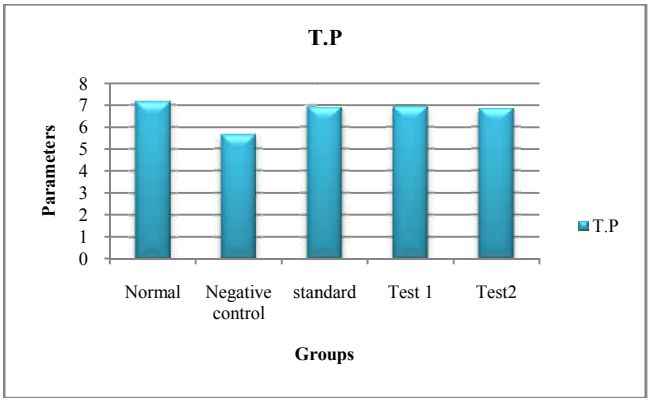

Figure 5 Effect of HALAV on TP

Effect of Hydro Alcoholic Leaf Extract of "Adhatoda vasica" on Antioxidant Parameters

The hydro alcoholic leaf extract of "*Adhatoda vasica"*is given at the dose of 50mg/Kg and 100mg/Kg and the antioxidant parameters are estimated and the values obtained are almost equal to that of standard. The antioxidant values-

Table 2 Effect of HALAV on SOD and LOP

Animals	SOD	LOP
Normal	30.42±0.95	12.61±0.34
Negative control	21.09±0.62	24.65±0.35
Standard	29.34±0.61	13.52±0.31
Test 1	26.12±0.11	16.92±0.78
Test 2	28.79±0.60	14.01±0.28

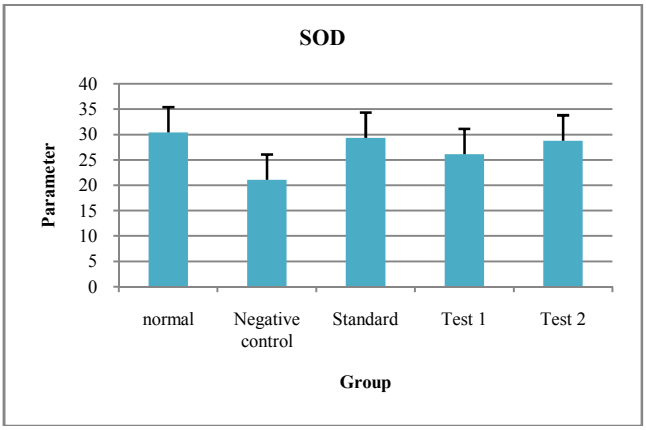

Figure 6 Effect of HALAV on SOD

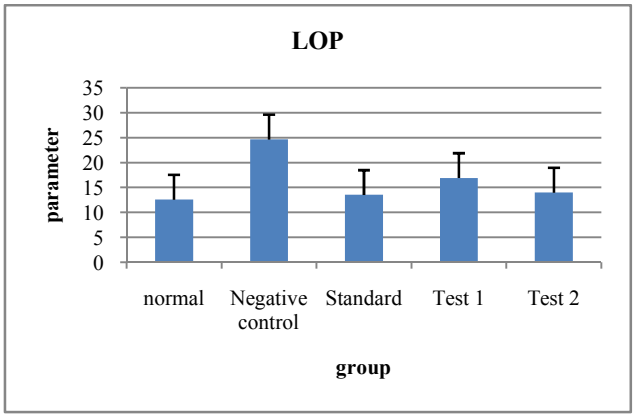

Figure 7 Effect of HALAV on SOD

Light Microscopic Evaluation

The liver was isolated and the histopathological studies were performed. It was found that

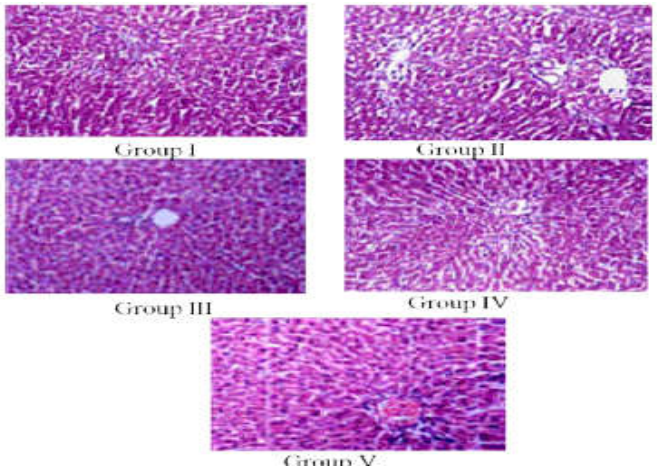

Figure 8 Histopathology of liver sections of animals (x100)

Group-I: olive oil (vehicle)

Group II: carbon tetra chloride treated group showing centrilobular degeneration, necrosis of hepatic cells,

Group III: carbon tetra chloride + Silymarin 100 mg/kg showing complete regeneration and normal architecture of hepatocytes.

Group IV: carbon tetra chloride + HALAV 50 mg/kg had shown mild degeneration and reverting to regeneration.

Group V: carbon tetra chloride + HALAV 100 mg/kg had shown maximum regeneration and almost near to normal architecture of hepatocytes

DISCUSSION AND CONCLUSION

The present examination uncovers that the hydro-alcoholic leaf concentrate of *Adhathoda vasica* display a critical Hepatoprotective movement by decreasing the biochemical parameters like AST, ALT and ALP when contrasted with the standard. The plant concentrates of 50 and 100 mg/kg has diminished the serum protein and aggregate bilirubin levels, lipid peroxidation levels and expanded the defensive Superoxide Dismutase levels too. In comparision with the standard medication Sylimarin both the centralizations of the concentrate demonstrated a critical hepatoprotective action. The hydro-alcoholic leaf concentrate of *Adhathoda vasica* has demonstrated a promosing hepatoprotective movement and further examinations are required to investigate the dynamic fixing in charge of the Hepato security and deal with the dangerous impacts of numerous medications.

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