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HEPATOPROTECTIVE ACTIVITY OF AQUEOUS METHANOLIC EXTRACT OF LEAVES OF *BASELLA RUBRA* L. AGAINST CCl_4 - INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for liver injury. The present study was conducted to evaluate the hepatoprotective activity of aqueous methanolic extract of leaves of *Basella rubra* in wistar rats. The study was conducted using carbon tetrachloride (2 ml/kg). Silymarin (50 mg/kg, p.o.) was used as reference drug in the respective model. The aqueous methanolic extract of *Basella rubra* has shown very significant hepatoprotection against and CCl_4-induced hepatotoxicity study model in wistar rats. This was evidenced by marked reduction in marker enzymes in serum.

INTRODUCTION:

Liver is the vital organ of metabolism and excretion. Hepatic injury is associated with distortion of metabolic functions, thus liver ailments remain as one of the serious health problems [(1)]. Drug induced liver injury is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and regulatory agencies. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all liver failure [(2)]. Chemicals frequently cause subclinical injury to liver that can be detected by estimating liver enzyme levels. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. However, there are not enough drugs available for the treatment of liver disorders. Recently, many folk remedies from plant origin are being evaluated for its possible antioxidant and hepatoprotective effects against different chemical-induced liver damage in experimental animals [(3, 4)].

Basella rubra known as Malabar/Ceylon spinach is a climbing perennial plant belongs to basellaceae family. It has thick tender stems and the leaves are almost circular to ovate, alternate and short petiole. They are thick, succulent and colored from green to

purple. The leaves are used in catarrhal affection and to hasten suppuration. Decoction of the root relieves bilious vomiting [5]. In general, spinach leaves contain several active components including flavonoids exhibit antioxidative, antiproliferative and anti-inflammatory properties in biological system. Other species of spinach extracts have been demonstrated to exert numerous beneficial effects such as chemo and central nervous system protection, anticancer and antiaging[6]. Leaves are used as anthelmentic, demulcent, anti-inflammatory, antimalarial and analgesic. Flowers are useful for removal of kidney stones, gonorrhea and headache.

MATERIALS AND METHODS

Plant material

Leaves of plant of *Basella rubra* was collected during flowering season from Venkateswarapuram village, Kadapa district, Andhra Pradesh, India. The plant was authenticated by Dr. Madhava chetty, Taxonomist, S.V. University, Tirupati, India. The collected leaves were washed immediately and dried at room temperature for a month, powdered mechanically, sieved (10/44) and stored in air-tight containers.

Preparation of Extracts

About 2000 g of the powdered material was subjected to soxhlation and exhaustively extracted with 80% methanol for 48 h. The solvent was distilled off at low temperature under reduced pressure using rotary flash evaporator. The semisolid mass obtained was dried in an oven at 40OC, powdered, labeled as MEBR and stored in desiccator.

Chemicals

Carbon tetrachloride was procured from S.D. Fine Chemicals Ltd. (India). Silymarin was obtained as gift sample from Ranbaxy (Devas, India). Standard kit of SGPT, SGOT, ALP and bilirubin were obtained from Jain Scientific Industries, Moradabad, India. All other reagents used were of analytical grade.

Phytochemical investigation

Phytochemical tests were carried out to find out the presence of phytoconstituents viz flavanoids, saponins, glycosides, carbohydrates, phenols etc and the results are shown in Table: 1

Experimental Animals

Wistar rats (150-200 g) were used in this experiment. They were housed in standard cages by maintaining a temperature of 22 ± 2ºC at 12:12 hours light dark cycle. The animals were provided with pellet diet and water *ad libitum*. The experimental procedures were carried out in strict compliance with the ethical guidelines for investigations of experimental pain in conscious animal framed by the Animal Ethical Committee rules and regulations in this institute.

Acute toxicity studies

Acute oral toxicity studies were conducted to determine the LD_{50} cut off value (mg/kg body weight) as per the OECD 2006 Guideline – 423, Up and Down Procedure.

Biochemical estimation of markers of oxidative stress

SOD activity was determined according to previous report[7]. CAT activity was determined from the rate of decomposition of H_2O_2 by the reported method[8]. GPX activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of $H_2O_2$and NaN_3[9]. Glutathione reductase activity was assayed according to previous reports[10].

Assessment of hepatoprotective activity

A toxic dose or repeated doses of a known hepatotoxin such as carbon tetrachloride, paracetamol, thioacetamide, rifampicin, alcohol, D-galactosamine, allyl-alcohol etc., are administered to induce liver damage in experimental animals[11]. If the hepatotoxicity produced by the toxin is prevented or reduced, then the test substance is considered as an effective hepatoprotective agent. In the present investigation, rats (n=6) were randomized into following groups and the pharmacological investigation was carried using carbon tetrachloride as inducing agent and the test MEBR at dose levels of 100, 200, 400 mg/kg as hepatoprotective agent.

1) Group I - 1% w/v CMC p.o.

2) Group II - CCl_4 (2 ml / kg) administered by i.p + 1% w/v CMC p.o.

3) Group III- CCl_4 (2 ml / kg) administered by i.p + MEBR (100 mg/kg) in 1% w/v CMC p.o.

4) Group IV- CCl_4 (2 ml / kg) administered by i.p + MEBR (200 mg/kg) in 1% w/v CMC p.o.

5) Group V - CCl_4 (2 ml / kg) administered by i.p + MEBR (400 mg/kg) in 1% w/v CMC p.o.

6) Group VI- CCl_4 (2 ml / kg) administered by i.p + Silymarin (50 mg/kg) in 1% w/v CMC p.o.

Treatment with plant extract was started after 24 h of administration of inducing agent. After 21 days of such treatment, rats were sacrificed by cervical dislocation. Blood was collected and serum was separated by allowing the blood samples to coagulate for 30 min at 37^{o}C followed by centrifugation (3000 rpm for 15 min) and subjected for determination of biochemical parameters like total bilirubin, SGPT, SGOT[12]. Liver was dissected out, washed with ice cold Phosphate Buffer Saline (PBS) (0.1 M, pH 7.4) and 10% tissue homogenate used to estimate serum enzyme levels.

STASTICAL ANALYSIS

The results are expressed as Mean ± SEM of six animals from each group. The data were evaluated by one-way ANOVA followed by Tukey's multiple comparison tests. *P values <0.05 was considered statistically significant.

RESULTS

Preliminary phytochemical screening

The various phytoconstituents present in the extract was given in Table 1. MEBR showed significant amounts of flavanoids and triterpenes.

Acute toxicity studies

The MEBR did not exhibit any toxic effects up to 5000 mg/kg body weight on oral administration. Body weight before and after administration were noted and any changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system, somatomotor activity, behavioral pattern were observed, sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were seen. The onset of toxicity and signs of toxicity were not seen in the rats up to 72 h of observation period. This indicates the safety of extract.

Biochemical parameters: Rats treated with carbon tetrachloride showed a significant hepatic damage as observed from elevated levels of hepato-specific enzymes as well as severe alteration in different liver parameters. SGPT(AST), SGOT(ALT), and total bilirubin in serum were increased in carbon tetrachloride intoxicated control animals. Treatment with the methanolic extract of *Basella rubra* caused significant protection against CCl_4–induced increase in serum enzyme levels and bilirubin in a dose responsive manner. Similarly, SOD, CAT, and GSH

were estimated from liver homogenate and against CCl_4 induced liver damage. MEBR showed significant protection

Table - 1: Qualitative phytochemical analysis of methanolic extract of *Basella rubra* (MEBR)

Constituent	MEBR
Alkaloids	
Mayer`s test	-
Dragendorff`s test	-
Wagner`s test	-
Hager`s test	-
Carbohydrates	
Molisch`s test	++
Fehling`s test	++
Benedict`stest	++
Phytosteroids	
Libermann-Burchard test	+++
Salkowski test	+++
Saponins	
Foam test	++
Flavonoids	
Ferric chloride test	+++
Shinoda test	+++
Lead acetate test	+
Alkaline reagent test	-

+++ High, ++ Moderate, + Slight, - Negative

TABLE 2**:** The effect of MEBR on serum enzymatic activity in CCl_4 - induced hepatotoxicity in rats.

GROUP	AST-- IU/L	ALT-- IU/L	ALP (KA Units)
Group I	35.12 ± 1.71*	35.74 ± 1.11*	296.71 ± 15.7*
Group II	76.38 ± 2.98*	129.31 ± 2.95*	839.17 ± 19.6*
Group III	59.36 ± 2.58*	82.9 ± 2.96*	665.71 ± 13.9*
Group IV	47.69 ± 2.14*	66.87 ± 1.85*	489.78 ± 17.24
Group V	43.29 ± 2.58	49.26 ± 1.58*	386.39 ± 9.45*
Group VI	37.38 ± 2.14*	43.47 ± 1.29*	358.27 ± 8.25*

The results are expressed as Mean ± SEM of six animals from each group. The data were evaluated by one-way ANOVA followed by Tukey's multiple comparison tests. *P values <0.05 was considered statistically significant. MEBR= methanolic extract of *Basella rubra*

TABLE 3**:** The effect of MEBR on SOD, CAT, and GSH in CCl_4 - induced hepatotoxicity in rats.

GROUP	SOD	CAT	GSH
Group I	14.98 ± 1.57	114.27 ± 4.25	4.36 ± 0.17
Group II	8.42 ± 0.32	49.68 ± 3.57	1.87 ± 0.57
Group III	9.78 ± 1.53	86.58 ± 4.38**	2.37 ± 0.17
Group IV	10.98 ± 1.59	97.25 ± 1.68	3.04 ± 0.19
Group V	11.95 ± 1.03	103.89 ± 1.32	3.51 ± 0.045**
Group VI	13.46 ± 0.71	109.18 ± 1.65	3.91 ± 0.13

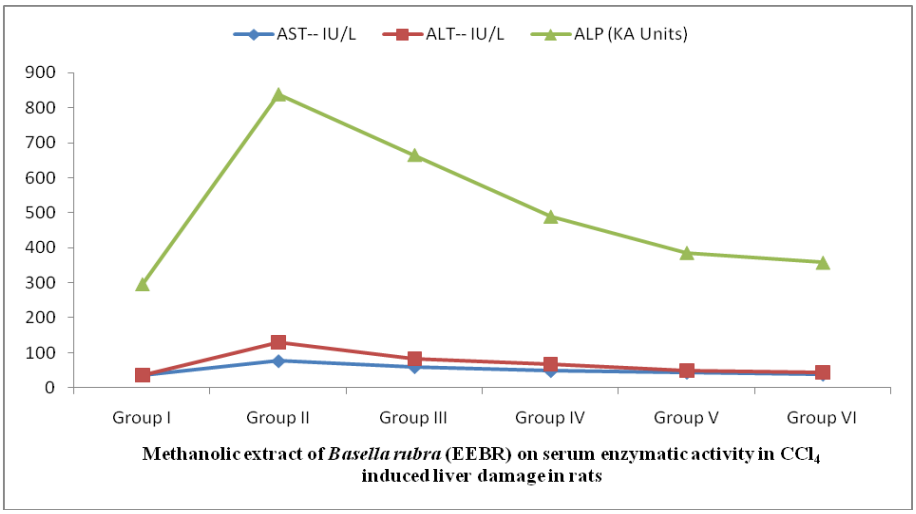

Fig:1 The effect of MEBR on serum enzymatic activity in CCl_4 - induced hepatotoxicity in rats.

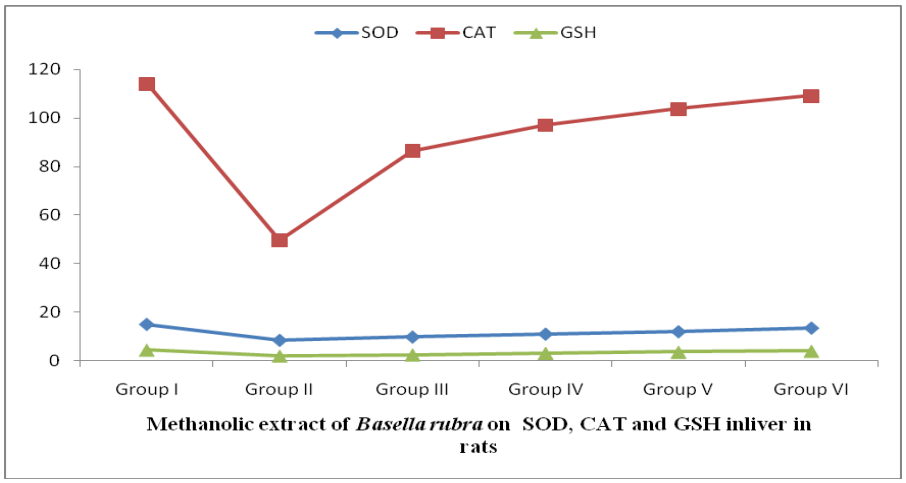

Fig: 2 The effect of MEBR on SOD, CAT, and GSH in CCl_4 - induced hepatotoxicity in rat

DISCUSSION

Carbon tetrachloride is widely used to induce acute-toxic liver injury in laboratory animals. The changes associated with CCl_4-induced hepatic damage are similar to that of acute viral hepatitis. The hepatotoxicity of CCl_4 has been reported to be due to its biotransformation by cytochrome P_{450} system to produce trichloroethylene free radicals. These free radicals may again react with oxygen to form trichloroethylene peroxy radicals, which exert their action on lipids membrane of endoplasmic reticulum to evoke lipid peroxidation[13]. In the present investigation, CCl_4 administration resulted in elevated activities of AST, ALT and ALP in serum against their respective control values. Similarly, serum bilirubin level was also found to be increased significantly as a result of CCl_4 toxicity. Oral administration of various doses of MEBR to CCl_4 intoxicated rats resulted in gradual normalization of the activities of AST, ALT and ALP. This evidently suggests the protective effect of the extract in improving the functional integrity of liver cells. Serum bilirubin is considered as an index for the assessment of hepatic function and any abnormal increase indicates hepatobiliary disease and severe disturbance of hepatocellular architecture. CCl_4 administration resulted in increased serum bilirubin level, thereby suggesting

severe hepatic injury and confirming the hepatotoxic nature of CCl_4. Treatment with MEBR significantly decreased the elevated level of total bilirubin in serum towards normalcy indicating its hepatoprotective efficacy. The curative efficacy of MEBR was dose dependent as evidenced by gradual reversal of the altered values of various biochemical markers back to normal following oral administration. This may, probably be though promotional activation of antioxidative enzymes and regeneration of hepatocytes that restore the structural and functional integrity of liver. The protective effects due to treatment with *Basella rubra* extract strongly indicated the possibility of the extract being able to prevent and/or mitigate any leakages of marker enzymes into circulation, condition the hepatocytes to accelerate regeneration of parenchymal cells, and preserve the integrity of the plasma membranes and hence restore these enzymes levels[(14)]. Thus, the present investigation confirms the hepatoprotective action of *Basella rubra* against CCl_4 induced hepatotoxicity in rats.

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