Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 5, 2011

Research Article

HEPATOPROTECTIVE ACTIVITY OF AQUEOUS ETHANOLIC EXTRACT OF AERIAL PARTS OF *BASELLA RUBRA* LINN AGAINST CARBON TETRACHLORIDE AND PARACETAMOL -INDUCED HEPATOTOXICITY IN RATS

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Received: 11 Aug 2011, Revised and Accepted: 11 Nov 2011

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 ethanolic extract of aerial parts of *Basella rubra* in wistar rats. The studies were conducted using the two popular inducing agents Paracetamol (2gm/kg,p.o.) in 1% CMC and Carbon tetrachloride (2 ml/kg). N-acetyl l-cystine (100mg/kg b.w) and Silymarin (50mg/kg, p.o.) were used as reference drugs in the respective models. The effect was estimated by measuring the enzymatic levels and histo- pathological studies. The aqueous ethanolic extract of aerial parts of *Basella ru*bra has shown very significant hepatoprotection against both Paracetamol induced and CCl4-induced hepatotoxicity study models in wistar rats. This was evidenced by marked reduction in marker enzymes in serum. Histopathological studies also confirmed the hepatoprotective nature of the extract.

Keywords: *Basella rubra,* Hepatoprotective, Paracetamol, Carbon tetrachloride, Flavanoids, Triterpenes.

INTRODUCTION

Liver is the vital organ of metabolism and excretion. It is continuously and variedly exposed to xenobiotics because of its strategic placement in the body. 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, 3).

More than 900 drugs have been concerned in causing injury to liver and it is the most familiar reason for a medicine to be quitted from the market. According to the United States Acute Liver Failure Study Group, drug-induced liver injury accounts for more than 50% of acute liver failure, including liver damage caused by overdose of acetaminophen and idiosyncratic liver injury triggered by other drugs. 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 [(4, 5)].

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 petioled. They are thick, rugose, succulent and coloured from green to purple. The leaves are used in catarrhal affection and to hasten suppuration. Decoction of the root relieves bilious vomiting [(6)]. 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 [(7)]. 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

Aerial parts of plant of *Basella rubra* was collected during flowering season from Utukur village, Kadapa district, Andhra Pradesh, India. The plant was authenticated by Dr.Madhava chetty, Taxonomist, S.V. University, Tirupathi, India. The collected plant was washed immediately and dried at 50° C for a week, 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% ethanol for 48 h. The solvent was distilled off at low temperature under reduced pressure using rotary flash evaporator (buchi, flawil, switzerland). The semisolid mass obtained was dried in an oven at 40°C, powdered, labeled as EEBR and stored in desiccator.

Chemicals

Carbon tetrachloride was procured from S.D. Fine Chemicals Ltd. (India). Silymarin and N-acetyl l-cystine were obtained as gift sample from Ranbaxy (Devas, India). Standard kit of SGPT, SGOT, ALP and bilirubin was 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± 2°C 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 LD50 cut off value (mg/kg body weight) as per the OECD 2006 Guideline – 423 and OPPT Up and Down Procedure.

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[8, 9]. If the hepatotoxicity produced by the toxin is prevented or reduced, then the test substance is considered as an effective hepatoprotective agent [10, 11]. In the present investigation, rats (n=6) were randomized into following groups and the pharmacological investigation was carried using carbon tetrachloride and paracetamol as inducing agents and the test EEBR at dose levels of 100, 200, 400 mg/kg as hepatoprotective agent.

1) Group I -1% w/v CMC perorally for 21 days.

2) Group II - CCl4 (2 ml / kg) administered by i.p + 1% w/v CMC perorally for 21 days.

3) Group III - Paracetamol (2gm/kg) in 1% CMC perorally for 21 days.

4) Group IV-CCl4 (2 ml / kg) administered by i.p + EEBR (100mg/kg) in 1% w/v CMC perorally for 21 days.

5) Group V- CCl4 (2 ml / kg) administered by i.p + EEBR (200mg/kg) in 1% w/v CMC per orally for 21 days.

6) Group VI- CCl4 (2 ml / kg) administered by i.p + EEBR (400mg/kg) in 1% w/v CMC per orally for 21 days.

7) Group VII- Paracetamol (2gm/kg) and EEBR (100mg/kg) in 1% w/v CMC perorally for 21 days.

8) Group VIII- Paracetamol (2gm/kg) and EEBR (200mg/kg) in 1% w/v CMC perorally for 21 days.

9) Group IX- Paracetamol (2gm/kg) and EEBR (400mg/kg) in 1% w/v CMC perorally for 21 days.

10) Group X - CCl4 (2 ml / kg) administered by i.p + Silymarin (50mg/kg) in 1% w/v CMC perorally for 7 days.

11) Group XI-Paracetamol (2gm/kg) and N-acetyl l-cystine (100mg/kg) in 1% w/v CMC perorally for 21 days.

Treatment with plant extract was started after 24 hrs of administration of inducing agents. 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 [12], 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 for different biochemical analysis. A part of the liver was used for histopathological studies.

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 different extracts were given in Table 1. EEBR showed significant amounts of flavanoids and triterpenes.

Acute toxicity studies

The EEBR 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 hr of observation period. This indicates the safety of extract.

Biochemical parameters

Rats treated with carbon tetrachloride and paracetamol showed a significant hepatic damage as observed from elevated levels of hepato-specific enzymes as well as severe alteration in different liver parameters. SGPT, SGOT, and total bilirubin in serum were increased in carbon tetrachloride intoxicated control animals. Treatment with the ethanolic extract of *Basella rubra* caused significant protection against paracetamol and CCl4–induced increase in serum enzyme levels and bilirubin in a dose responsive manner. Similarly, LP, SOD, CAT, GSH and glycogen contents were estimated from liver homogenate and EEBR showed significant protection against both paracetamol and CCl4 induced liver damage.

Histopathological Studies

Histhopatological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central veins. Disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration were observed in paracetamol and CCl4 intoxicated animals. The liver sections of the rats treated with aqueous ethanolic extract of *basella rubra* and standard drugs followed by paracetamol and CCl4 intoxication showed a sign of protection as it was evident the absence of necrosis and vacuoles.

DISCUSSION

Carbon tetrachloride and paracetamol are the well known hepato-destructive agents that are widely used to induce acute-toxic liver injury in laboratory animals [13]. The changes associated with CCl4-induced hepatic damage are similar to that of acute viral hepatitis [14]. The hepatotoxicity of CCl4 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 [15]. Overdose of paracetamol causes a potentially fatal, hepatic centrilobular necrosis. The hepatotoxicity of paracetamol has been attributed to the formation of a toxic metabolite, *N-acetyl-p-benzoquinoneimine* (NAPQI) by the action of cytochrome P4502E1 [16].

In the present investigation, CCl4 and paracetamol 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 CCl4 and paracetamol toxicity. On the other hand, total serum protein level was lowered in response to CCl4 and paracetamol administration when compared with control. Abnormally higher activities of serum ALT, AST and ALP after CCl4 and paracetamol administration are an indication of the development of hepatic injury, which is responsible for leakage of cellular enzymes into the blood. When liver plasma membrane gets damaged, a variety of enzymes normally located in the cytosol are released into the circulation [17].

Oral administration of various doses of EEBR to CCl4 and paracetamol 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 [18]. CCl4 and Paracetamol administration resulted in increased serum bilirubin level, thereby suggesting severe hepatic injury and confirming the hepatotoxic nature of CCl4 and paracetamol. Treatment with EEBR significantly decreased the elevated level of total bilirubin in serum towards normalcy indicating its hepatoprotective efficacy.

Hepatotoxins impair the capacity of liver to synthesize albumin. Decreased total serum protein level in CCl4 and paracetamol treated rats may be attributed to impaired protein synthesis by damaging liver tissue. Subsequent treatment of CCl4 and paracetamol intoxicated rats with EEBR increased the total serum protein (TSP) level. This further signifies the curative nature of extract against CCl4 and paracetamol toxicity.

Hepatic lipid peroxidation (LP), expressed as TBARS (thiobarbituric acid reacting substances), increased significantly in CCl4 and paracetamol toxicity. While, the activities of protective enzymes such as Superoxide dismutase (SOD) and catalase (CAT) and glutathione and glycogen content in liver tissue were lowered after paracetamol administration. Enhanced LP and reduced activities of SOD and CAT is an indication of generation of free radical stress as a mark of hepatic damage due to CCl4 and paracetamol toxicity. Marked reductions in the activities of these free radical scavenging enzymes, SOD and CAT, associated with CCl4 and paracetamol toxicity were significantly reversed to normal on oral feeding of EEBR in a dose dependent manner conferring the antilipid peroxidative ability to the extract.

Paracetamol gets metabolically activated to a reactive metabolite NAPQI by cytochrome P4502E1[16] (Lee et al., 1996). NAPQI, in turn, is detoxified by conjugating with glutathione (GSH). Thus, GSH constitute the first line of defence against paracetamol induced generation of free radicals [18] (James. et al., 2003). In paracetamol toxicity, total hepatic GSH was found to be depleted due to the damage caused to hepatic cells. As a result, formation of NAPQI glutathione conjugate is diminished. Administration of EEBR effectively replenished the paracetamol induced depletion of hepatic GSH presumably due to diminished production of toxic metabolite, NAPQI through the inhibition of cytoP450 enzyme system.

CCl4 and Paracetamol induced damage of hepatocytes is also a reason behind decreased glycogen content of liver tissue. Significant increase in hepatic glycogen level was observed after administration of the extract indicating improvement in hepatic status. Histopathological examination of liver sections of the normal control group showed normal cellular architecture with distinct hepatic cells. However, distinct hepatic necrosis was noted after CCl4 and paracetamol administration with destruction of hepatic cells. *EEBR* treatment to such CCl4 and paracetamol intoxicated rats showed recovery of the hepatocytes from necrosis. This also suggests that the plant extract has a tremendous potential to reverse the changes induced by paracetamol toxicity back to normal.

The curative efficacy of EEBR 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 through 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 [20]. Thus, the present investigation confirms the hepatoprotective action of *basella rubra* against paracetamol induced hepatotoxicity in rats.

ACKNOWLEDGEMENT

We are thankful to Management of P. Rami Reddy memorial College of Pharmacy, Kadapa, A.P for the providing the all facilities for carried out this work.

Table 1: Qualitative phytochemical analysis of ethanolic extract of *Basella rubra*

Constituent	EEBR
Alkaloids	
Mayer`s test	-
Dragendorff`s test	-
Wagner`s test	-
Hager`s test	-
Carbohydrates	
Molish`s test	+++
Fehling`s test	++
Benedict`stest	++
Glycosides	
Libermann-Burchard test	+++
Salkowski test	+++
Borntrager`s test	-
Saponins	
Foam test	+++
Phenolic compounds	
Ferric chloride test	++
Shinoda test	+++
Lead acetate test	+
Alkaline reagent test	-

(EEBR) +++ High, ++ Moderate, + Slight, - Negative

Table 2: Ethanolic extract of *Basella rubra* (EEBR) on serum enzymatic activity in CCl4 and Paracetamol induced liver damage in rats (n = 6)

Group	AST-- IU/L	ALT-- IU/L	ALP (KA Units)	Total Proteins (mg/dl)	Bilirubin (mg%) (Total)
Group I	34.22 ± 1.60*	36.75 ± 1.09*	292.66± 14.9*	192 ±10.99*	0.29±0.01
Group II	72.33 ± 3.24*	135.22 ± 3.1*	841.22± 21.6*	68.7 ± 14.19*	4.58±0.16
Group III	70.27 ± 2.45*	113.8 ± 2.31*	892.36± 18.24*	72.4 ± 10.2*	3.92±0.25
Group IV	57.23 ±1.34*	81.33 ± 3.54*	652.2± 14.3*	88.4 ± 13.99*	1.73±0.21
Group V	45.45 ± 2.15*	64.34 ± 2.43*	497.7± 19.2	116.7 ± 14.56*	1.09±0.11
Group VI	41.36 ± 1.82	48.45 ± 1.34*	382.3±	156.6±	0.67±0.07
Group VII	56.12 ±1.12*	77.51 ± 2.36*	617.27± 24.38*	96.2 ± 8.64*	1.64±0.25
Group VIII	44.4 ± 1.15*	59.71 ± 1.14*	511.3± 16.5*	124.9 ± 12.6*	1.22±0.18
Group IX	40.32 ± 1.32	47.81 ± 1.78*	412.6± 10.5*	161.3± 9.28*	0.71±0.13
Group X	38.16 ± 1.37*	42.35 ± 1.41*	359.9± 10.2*	179.6 ± 13.02*	0.48±0.16
Group XI	36.48 ± 1.24*	39.97 ± 1.44*	381.5± 15.2*	183.6 ± 8.62*	0.52±0.16

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. EEBR= ethanolic extract of *Basella rubra*

Table 3: Ethanolic extract of *Basella rubra* (EEBR) on LP, SOD, CAT, GSH and Glycogen content in liver in rats (n = 6)

Group	LP (ŋ moles of MDA formed/mg protein)	SOD (Units of activity/mg protein)	CAT (µ moles of H2O2 decomposed /mg protein)	GSH (µg/mg protein)	Glycogen (mg/gm of wet tissue)
Group I	28.16 ±0.81	15.68±1.22	116.24±2.25	4.41±0.16	7.29±0.88
Group II	618.29 ±12.4*	8.6±0.67	50.87±3.69	1.92±0.62	4.62±1.06*
Group III	712.9 ±24.5	8.39±0.64	61.22±4.18	2.08±0.88*	5.22±0.84
Group IV	423.4 ±8.6	10.39±1.81	67.2±3.91	3.16±0.26**	6.21±0.38
Group V	280.32±4.22**	11.81±1.06	81.55±1.68	3.72±0.12	6.88±0.35
Group VI	164.3 ±6.28	12.54±1.02	93.26±2.46	3.96±0.14	6.93±0.29**
Group VII	463.9 ±18.4*	9.68±1.88	88.32±2.66**	2.17±0.11	5.99±0.92
Group VIII	267.9 ±11.2	11.04±1.72	96.48±1.99	2.94±0.09	6.42±0.44
Group IX	151.9 ±3.8**	12.84±1.04	102.22±1.44	3.61±0.05**	6.84±0.88
Group X	118.6 ±2.81**	13.52±0.92	106.24±1.85	3.82±0.09	7.11±0.26
Group XI	94.8 ±6.5**	12.96±0.64	107.12±1.08	4.01±0.06	7.06±0.09

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. EEBR= ethanolic extract of *Basella rubra*

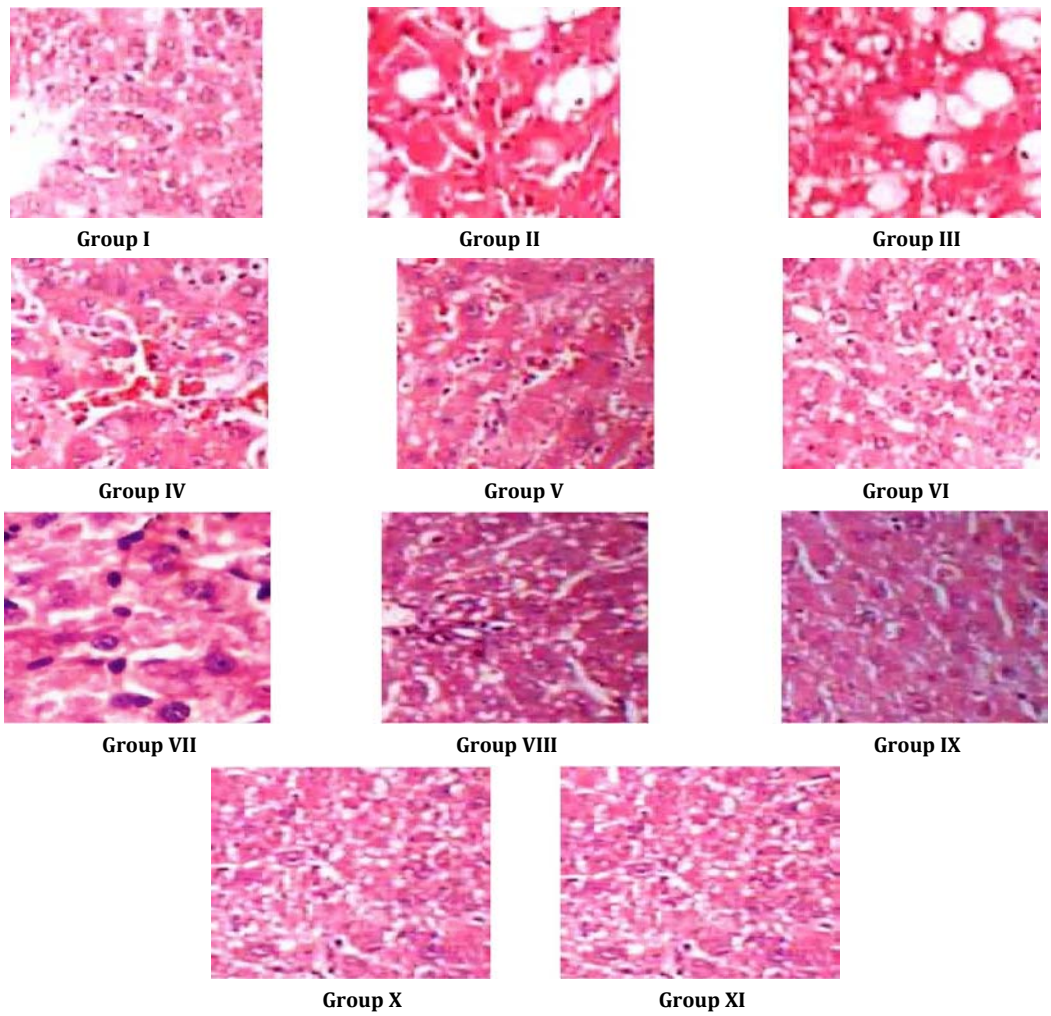

Fig. Microphotographs (10 x 40) of liver section taken from rats

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