ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 8, Issue 1, 2015

ISSN - 0974-2441

Research Article

HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *ROSTELLULARIA PROCUMBENS* BY USING ETHANOL INDUCED MODEL IN RATS

WILSON K, MANASA V, VIJUSHA M*, SUTHAKARAN R

Department of Pharmacology, Teegala Ram Reddy College of Pharmacy, Hyderabad, Telangana, India. Email: vijusha11@gmail.com

Received: 03 November 2014, Revised and Accepted: 11 November 2014

ABSTRACT

Objectives: *Rostelluria procumbens* is a medicinal plant used traditionally in the treatment of asthma, cough, and constipation and as an antioxidant, etc. it is rich in phytochemical compounds, which are responsible for its biological properties. This study focused on evaluation of hepatoprotective activity of methanolic extract of *R. procumbens* leaf in ethanol-induced hepatotoxocity in rats. In this study, 30 Wistar rats were used and grouped into five groups containing six rats each. **Methods:** In the study, ethanol is used as hepatotoxin. Four groups were treated with ethanol and taken as disease control, standard and two test groups. One group was taken as control treated with saline. Blood samples were collected and estimated serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase and total bilirubin which are key markers of liver function. **Results:** Upon oral administration of methanolic leaves extract of *R. procumbens* to ethanol intoxicated rats resulted in significant restoration of enzyme levels and also silymarin at a dose of 25 mg/kg. The reversal of increased serum enzymes in ethanol-induced liver damage by the extract may be due to the prevention of leakage of intracellular enzymes by its membrane stabilizing activity. **Conclusion:** The results confirm that *R. procumbens* have hepatoprotective activity against ethanol-induced hepatotoxicity and significant hepatoprotection seen at 500 mg/kg dose.

Keywords: *Rostellularia procumbens*, Hepatoprotective activity, Serum glutamate oxaloacetate transaminase, Serum glutamate pyruvate transaminase, Alkaline phosphatase, Total bilirubin, Ethanol.

INTRODUCTION

Herbal medicine is a triumph of popular therapeutic diversity [1]. The world is now moving towards the herbal medicine or system, which can then properly fight foreign invaders and help to destroy offending pathogens without toxic side-effects [2]. The world health organization in the early 1970's had encouraged government to effectively utilize local knowledge of herbal medicines for disease prevention and health promotion [3]. WHO has showed great interest in documenting the use of medicinal plants used by tribal's from different parts of the world [4]. Today this herbal medicine is coming into prominence because of the efficiency of the conventional medicines such as antibiotics, which have developed resistance to the many of the infectious organisms whereas herbs and its active constituents are being used to treat the infections, which no longer responsive to conventional medicine. The unique feature of traditional medicine in India is that it flourishes at multiple levels.

A herbal drug consists of a definite parts of single plant or mixture of plants which may be further processed through crushing, drying, powdering, etc., or extracting the juice either through pressure or by means of water at room temperature or by the application of heat. The product that is obtained is a very complex mixture of components that is belonging to different chemical classes the bio acting of which combined to give an effect, which is delivered from the synergistic or antagonistic effects of individual component.

The liver is the largest organ in the body weighing 1200-1500 g. It is a key organ in regulating homeostasis within the body. It regulates several important functions including protein synthesis, storage and metabolism of fats and carbohydrates, detoxification of drugs and other toxins, metabolism of hormones and excretion of bilirubin. Liver diseases are associated with distortion of these metabolic functions [5]. Although viruses are the main cause of liver diseases, the liver lesions arising from xenobiotics, excessive drug therapy, environmental pollution and alcoholic intoxication are not uncommon [6].

Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue glutathione levels. In addition, serum levels of many biochemical markers like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), triglycerides, cholesterol, bilirubin, alkaline phosphatase (ALP), are elevated [7,8].

MATERIALS AND METHODS

Plants are about 10-40 cm tall. Leaves are 2-4 cm long, 1-2 cm wide. Flowers in the form of inflorescences with 3-5 cm long, both bracts, bracteoles and calyx 5-7 mm long, pale purple (or merely white) corolla 7 mm long, 2 stamens, flowering in August-October. Fruits are 5-7 mm long. Annual plants. It widely distributed in open places at low and médium altitudes, from the Batan Islands and Northern Luzon to Mindanao, in most Islands and provinces and also in China and southward to Australia.

Collection and autentification of plant material

The plant material was collected in the month of June 2013 from Srichalam hills, and a specimen was dropped in the herbarium and the leaves were authenticated by Professor Dr. Madhavachetty S. V. University, Tirupathi. The collected powdered material was shade-dried and pulverized.

Solvents used for extraction: Petroleum ether and methanol.

Preparation of the extract

The dried powders of leaf of *Rostellularia procumbens* were defatted with petroleum ether (60-80°C) in a Soxhlet Apparatus by continuous hot-percolation. The defatted powder material (marc) thus obtained was further extracted with methanol with the same method. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried using rotary flash evaporator. The resultant dried extracts were used for further study.

Phytochemical screening

The screening was carried out in accordance with the standard protocol as described by Trease and Evans (1983).

Test for reducing sugars (Fehling's test)

The aqueous ethanol extract (0.5 g in 5 ml of water) of individual plants was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a color reaction.

Test for anthraquinones

The individual plant extract (0.5 g) was boiled with 10 ml of sulfuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube, and 1 ml of dilute ammonia was added. The resulting solution was observed for color changes.

Test for terpenoids (Salkowski test)

To 0.5 g each of the individual extracts was added 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration was confirmed for the presence of terpenoids.

Test for flavonoids

A portion of the individual plant extract (0.5 g) was heated with 10 ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered, and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids.

Test for saponins

To 0.5 g of each plant extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins

About 0.5 g of the individual extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride ($FeCl_3$) was added and observed for brownish green or a blue-black coloration.

Test for alkaloids

A total of 0.5 g of each extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test)

To 0.5 g of individual plant extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with 1 ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Acute oral toxicity study

Acute oral toxicity test was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for 3-4 hrs providing only water, after which the extracts were administered orally at the dose level of 5 mg/kg by intragastric tube and observed for 3 days. If the mortality was observed in 2-3 animals then, the dose administered was assigned as a toxic dose. If the mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If the mortality was not observed, the procedure was repeated for further higher doses such as 5000, 3000 and 2000 mg/kg.

Ethanol induced the hepatotoxicity model

Animals were divided into five groups of six animals each:

Group I: Treated as normal control and receives 5% gum acacia.

Group II: Treated as toxic control and is administered with ethanol to induce hepatotoxicity.

Group III: Administered with ethanol and standard drug silymarin at a dose of 25 mg/kg.

Group IV: Administered with ethanol and MERP extract at a dose of 250 mg/kg.

Group V: Administered with ethanol and MERP extract at a dose of 500 mg/kg.

After 30 minutes of ethanol administration Group III, IV and Group V rats were treated with Silymarin (25 mg/kg/day), MERP 250 mg/kg/day and MERP 500 mg/kg/day respectively. All the animals were sacrificed by cervical decapitation under light ether anesthesia on the 9th day. Blood was collected by heart puncture and centrifuged (300 rpm for 10 minutes) to obtain serum. The serum was used for the assay of total ALP, SGOT, SGPT and total bilirubin (TB). The liver was dissected out immediately after sacrifice, weighed and washed in ice-cold saline. Small pieces of liver tissue were collected and preserved in 10% formalin solution for histopathological studies.

Statistical analysis

The results obtained in biochemical assays were given in terms of mean±standard error mean. The statistical significance of the data was assessed by one-way analysis of variance (ANOVA) followed by Dunnett's t-test between different groups. Toxic, standard and test groups were compared with normal group. Standard and all test groups were compared with the toxic group. $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Phytochemical investigation

Phytochemical studies revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids and diterpenes while phytosterols, saponins, tannins, and proteins were absent in the extract.

There are many factors responsible for liver damage or injuries such as chemicals and drugs. In the present study, ethanol was used to induce hepatotoxicity since it is clinically relevant. Acute and chronic ethanol administration cause enhanced formation of cytokines especially tumor necrosis factor-α by hepatic Kuffer cells, which have a significant role in liver injury. Besides the development of fatty liver, another early

Table 1: Phytochemical investigation of methanolic leaves extract of *R. procumbens*

S.No	Constituents	Test	Methanolic extract
1	Alkaloids	Mayer's reagent Wagner's reagent Dragendroff's reagent Hagner's reagent	Present
2	Carbohydrates	Molisch's reagent Benedict's reagent Fehling's solution	Present
3	Glycosides	Modified Borntrager's test Legal test	Present
4	Phytosterol	Salkowski's test Libermann Burchard's test	Absent
5	Saponins	Froth test Foam test	Absent
6	Tannins	Gelatin test	Absent
7	Proteins	Xanthoprotein test Ninhydrin test	Absent
8	Flavonoids	Alkaline reagent test Lead acetate test Shinoda test	Present
9	Diterpenes	Copper acetate test	Present

R. procumbens: Rostellularia procumbens

Table 2: Effect of MERP on biochemical parameters on ethanol induced hepatotoxic rats

Groups	Treatment	SGPT (U/L)	SGOT U/L)	ALP (U/L)	TB mg/dL	Mean liver weight (g/100 g)
Group I	Control	42.83±1.65	63±1.07	141.33±2.49	0.61±0.32	6.66±0.67
Group II	Ethanol	147.66±1.76	233.5±1.83	289.66±3.18	2.47±0.3	8.83±1.17
Group III	Silymarin+ethanol	41±2.184**	70±1.53**	143.5±2.81**	0.57±0.06**	6.5±0.728**
Group IV	MERP 300 mg/kg+ethanol	53.66±1.548*	112.6±2.10*	181.16±1.840*	1.4±0.02*	7.8±0.634
Group V	MERP 500 mg/kg+ethanol	42.61±1.3**	82.4±1.42**	156.6±1.6**	0.73±0.02**	6.8±0.5**

SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, ALP: Alkaline phosphatase, TB: Total bilirubin, Each value is the mean±SEM for 6 rats, *p<0.5, **p<0.01, ***p<0.001

sign of excessive ethanol consumption is liver enlargement and protein accumulation, both of which are common findings in alcoholics and heavy drinkers.

Ethanol metabolized by three different ways
1. By enzyme alcohol dehydrogenase (ADH)
2. By CYP450 enzyme CYP2E1
3. By mitochondrial catalase.

ADH and CYP2E1 both convert ethanol to acetaldehyde, which is in part responsible for liver damage. However, the process of liver injury is much more complex resulting from biochemical, genetic, cellular, immunological changes in connection with the intake and metabolism of excessive quantities of ethanol (Table 1).

Biochemical parameters

In the present study MERP at 300 mg/kg and 500 mg/kg studied for hepatoprotective activity in ethanol-induced hepatotoxicity in rats. In general, measurement of SGPT, SGOT, ALP and TB are commonly used as marker enzymes of hepatotoxicity. The more specific cytosolic SGPT found in high concentration in the liver and SGOT, which is localized in the cytosol and mitochondria are released into circulation due to liver injury. Prolonged destruction of the hepatic cells results in more hepatic releases to exacerbate hepatic dysfunction and causes an elevation in the serum levels of ALP and TB [10] (Table 2).

On oral administration of methanolic leaves extract of *R. procumbens* to ethanol intoxicated rats resulted in significant restoration of enzyme levels and also silymarin at a dose of 25 mg/kg. The reversal of increased serum enzymes in ethanol-induced liver damage by the extract may be due to the prevention of leakage of intracellular enzymes by its membrane stabilizing activity.

Normally the serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes. Based on the present study analysis, it is known that *R. procumbens* has hepatoprotective activity, which may gradually increase with the dose.

CONCLUSION

The preliminary phytochemical studies reveal the presence of flavonoids. The flavonoids are known as hepatoprotectives, so the observed hepatoprotective activity of *R. procumbens* may be due to the presence of flavonoids. The mechanism of hepatoprotective activity may be due to activation of regeneration of hepatocytes that restores the structural and functional integrity of liver. Further studies to characterize the active principles and to elucidate the mechanism are in progress.

REFERENCES

1. Shu YZ. Recent natural products based drug development: A pharmaceutical industry perspective. J Nat Prod 1998;61:1053-71.
2. Pandey M, Debnath M, Gupta S, Chikara SK. Phytomedicine an ancient approach turning into future potential source of therapeutics. J Pharmacogn Phytother 2011;3(3):27-37.
3. Ravishankar B, Shukla VJ. Indian systems of medicine: A brief profile. Afr J Tradit Complement Altern Med 2007;4(3):319-37.
4. Kaido TL, Veale DJ, Havlik I, Rama DB. Preliminary screening of plants used in South Africa as traditional herbal remedies during pregnancy and labour. J Ethnopharmacol 1997;55(3):185-91.
5. Ward FM, Daly MJ. Liver disease. In: Walker R, Edwards C, editors. Clinical Pharmacy and Therapeutics. 3rd ed. New York: Churchill Livingstone; 2005. p. 209.
6. Rajan BG, Chezhiyan N. Strength and wealth of therapeutic medicinal plants in India. In: Khan IA, Khanum A, editors. Role of Biotechnology in Medicinal and Aromatic Plants, Special Volume on Diseases. Vol. 6. Hyderabad: Ukaaz Publications; 2002. p. 151-2.
7. Mossa JS, Tariq M, Mohsin A, Ageel AM, al-Yahya MA, al-Said MS, *et al*. Pharmacological studies on aerial parts of *Calotropis procera*. Am J Chin Med 1991;19(3-4):223-31.
8. Mascolo N, Sharma R, Jain SC, Capasso F. Ethnopharmacology of *Calotropis procera* flowers. J Ethnopharmacol 1988;22(2):211-21.
9. Balne D, Pallerla P, Vanapatla SR, Bobbala RK. Hepatoprotective effect of whole plant extract fractions of *Marsilea minuta* linn. Asian J Pharm Clin Res 2013;6(3):100-7.
10. Kshirsagar AD, Mohiter R, Aggarwal AS, Suralkar UR. Hepaoprotactive medicinal plants of ayuveda. Asian J Pharm Clin Res 2013;4(3):1-8.