

Hepatoprotective activity of methanolic leaves extract of *Rostellularia procumbens* by using carbon tetrachloride intoxicated rats

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Received: 20 October 2014

Accepted: 07 November 2014

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ABSTRACT

Background: *Rostellularia 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. The present study focused on evaluation of hepatoprotective activity of methanolic extract of *R. procumbens* leaf in carbon tetrachloride (CCl₄) induced hepatotoxicity in rats.

Methods: In this study, 30 wistar rats were used and grouped into 6 each group contain 6 rats. In this study, CCl₄ is used as hepatotoxin. Four groups were treated with CCl₄ 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. The rats were sacrificed and livers were isolated and histopathological studies carried out.

Results: 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.

Conclusion: The results confirm that *R. procumbens* have hepatoprotective activity against CCl₄ 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, Carbon tetrachloride

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.

An herbal drug consists of 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 such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), triglycerides, cholesterol, bilirubin, alkaline phosphatase (ALP), are elevated.^{7,8}

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, two stamens, flowering in August-October. Fruits are 5-7 mm long.

Collection and authentication 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 sulphuric acid (H₂SO₄) 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 H₂SO₄ (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 mins. 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 were 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 Dragendorff's reagent to the other. The formation of cream (with Mayer's reagent) or reddish brown precipitate (with Dragendorff'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 sulfuric acid. 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 mortality was observed in 2-3 animals then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg.

Hepatoprotective activity

Screening methods for hepatoprotective activity

1. Paracetamol induced the hepatotoxicity model
2. Arsenic induced the hepatotoxicity model
3. Isoniazide induced hepatotoxicity model
4. Carbon tetrachloride (CCl₄) induced hepatotoxicity model
5. Glucocorticoids induced hepatotoxicity model
6. Nonsteroidal anti-inflammatory drugs associated toxicity of the liver.⁹

Models chosen for the study:

CCl₄ induced hepatotoxicity model.

CCl₄ induced hepatotoxicity

Rats were divided into five groups each contains six rats. Group I (control) animals were administered a single dose of 5% gum acacia suspension (1 ml/kg, p.o) daily for 5 days and received liquid paraffin (1 ml/kg, s.c) on day 2 and 3. Group II (toxic control) received 5% gum acacia suspension (1 ml/kg, p.o) daily for 5 days and received CCl₄: liquid paraffin (1:1, 2 ml/kg, s.c) on day 2 and 3. Group III (standard) received silymarin (25 mg/kg, p.o). Group IV and Group V (test groups) were administered orally a dose of MERP 250 mg/kg and 500 mg/kg daily for 5 days respectively. Groups III-V animals were administered simultaneously CCl₄: liquid paraffin (1:1, 2 ml/kg, s.c) on day 2 and 3 after administration of silymarin and extracts. Animals were scarified 24 hrs after the last treatment. Blood was collected, allowed to clot and serum was separated at 2500 rpm for 15 mins and biochemical investigations

were carried out. Liver was dissected out and used for histopathological studies.

Biochemical investigations

The biochemical parameters like serum enzymes like SGOT, SGPT, ALP total bilirubin (TB) and total protein levels were assayed using assay kits.

Histopathological studies

Histopathological studies could be carried out to assess the degree of damage. This is done by staining the fine section of liver isolates and examining under a microscope. After the animals had been sacrificed, livers were taken out and washed with normal saline (0.9%). Then, 2-3 pieces of approximately 6 cu.mm size were cut and fixed in phosphate-buffered 10% formaldehyde solution. After embedding in paraffin wax, thin sections of 5 µm thickness of liver tissue were cut and stained with hemotoxylin-eosin stain.

Statistical analysis

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

RESULTS

Phytochemical investigations

Extract subjected for phytochemical investigation showed the presence of alkaloids, carbohydrates, glycosides, flavanoids and diterpenes (Table 1).

Acute toxicity studies

The methanolic extract did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose.

Biochemical investigations

The results of hepatoprotective activity of the extract in CCl₄ intoxicated rats are shown in Table 2. In CCl₄ intoxicated Group II serum SGPT, SGOT, ALP and TB were increased up to 62 U/ml, 83 U/ml, 246.3 IU/L and 5.19 mg/dl respectively, whereas the values were 28.16 U/ml, 28.166 U/ml, 110 IU/L and 1.19 mg/dl in control Group I respectively (Table 2).

The elevated levels of serum SGPT, SGOT, ALP and TB were significantly reduced in the animals treated with an

extract at 250 mg/kg and 500 mg/kg doses. Treatment with 500 mg/kg dose showed significant activity ($p < 0.001$) with a maximum reduction in the serum enzyme levels and but not as effective as silymarin (Figures 1-4).

Histopathological studies

Histopathological examination of liver sections of the control group showed normal hepatocytes, multiple FAN are seen with mild portal tract infiltration with lymphocytes (Figure 5a). In CCl_4 treated animals, expanded portal tracts with lymphocyte infiltration and perivascular and periportal vacuolated and degenerated hepatocytes are seen, and extensive vacuolated (microvesicular) hepatocytes seen with perivascular inflammatory infiltrate of lymphocytes and neutrophils (Figure 5b). The liver sections of the rats treated

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

Constituents	Test	Methanolic extract
Alkaloids	Mayer's reagent Wagner's reagent Dragendroff's reagent Hagner's reagent	Present
Carbohydrates	Molisch's reagent Benedicts reagent Fehling's solution	Present
Glycosides	Modified Borntrager's test Legal test	Present
Phytosterol	Salkowski's test Liebermann Burchard's test	Absent
Saponins	Froth test Foam test	Absent
Tannins	Gelatin test	Absent
Proteins	Xanthoprotein test Ninhydrin test	Absent
Flavonoids	Alkaline reagent test Lead acetate test Shinoda test	Present
Diterpenes	Copper acetate test	Present

R. procumbens: *Rostelluria procumbens*

Table 2: Effect of *R. procumbens* leaves extract on biochemical parameters of rats.

Treatment	SGPT (U/ml)	SGOT (U/ml)	ALP (IU/L)	TB mg/dl
Group I Control	28.16±2.47	28.166±1.050	110±3.38	1.19±0.131
Group II CCl_4	62±1.53	83±1.94	246.3±2.52	5.19±0.190
Group III Silymarin+ CCl_4	24.66±1.234**	33.33±2.018**	142.33±1.77**	2.13±0.20**
Group IV MERP 300 mg/kg+ CCl_4	54.5±1.263	69±2.59	232.5±2.97	4.95±0.16
Group V MERP 500 mg/kg+ CCl_4	24±1.533**	37.33±1.898**	176.66±3.085*	3.16±0.15*

CCl_4 : Carbon tetrachloride, MERP: Methanolic leaves extract of *Rostellularia procumbens*, SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamate oxaloacetate transaminase, ALP: Alkaline phosphatase, TB: Total bilirubin, *R. procumbens*: *Rostelluria procumbens*

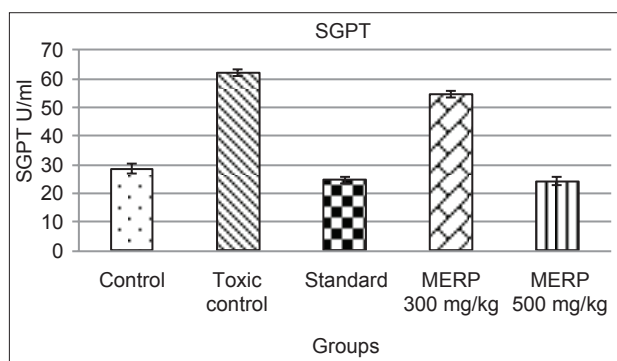


Figure 1: Effect of *Rostelluria procumbens* leaves extract on serum serum glutamate pyruvate transaminase levels in carbon tetrachloride treated rats.

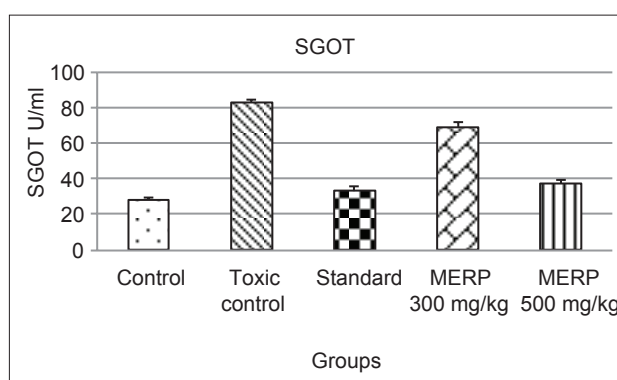


Figure 2: Effect of *Rostelluria procumbens* leaves extract on serum serum glutamate oxaloacetate transaminase levels in carbon tetrachloride treated rats.

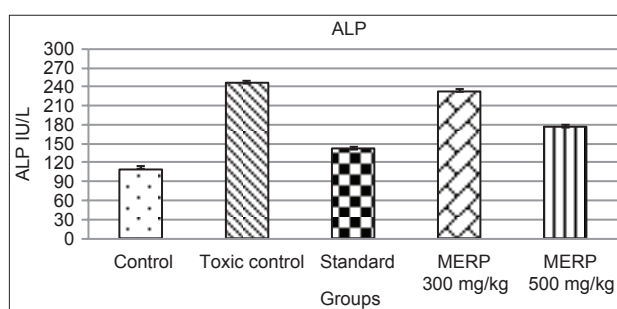


Figure 3: Effect of *Rostelluria procumbens* leaves extract on serum alkaline phosphatase levels in carbon tetrachloride treated rats.

with 250 mg/kg of *R. procumbens*, followed by CCl₄ showed multiple FAN with portal tract and perivascular lymphocyte infiltrate with perivascular and periportal degenerated and vacuolated hepatocytes. Perivascular and periportal small vacuolar change in hepatocytes is also seen (Figure 5d); and *R. procumbens* (500 mg/kg) and silymarin followed by CCl₄ showed a sign of protection as it was evident by multiple small FAN seen with mild to moderate portal tract infiltration with lymphocytes (Figures 5c and e) (Figure 5).

DISCUSSION

CCl₄ is one of the most commonly used hepatotoxin in the experimental study of liver diseases. CCl₄ causes altered permeability of membrane resulting in leakage of hepatic marker enzymes (aspartate transaminase [AST] [or] SGOT and alanine transaminase [ALT] [or] SGPT) from cells into circulation.¹⁰ Hence the elevation of levels of these enzymes in serum acts as a reliable marker for assessing hepatotoxicity. AST predominantly found in mitochondria of hepatocytes. ALT is more specific to liver and thus is a better parameter for detecting liver injury. Serum ALP, TB,

and total proteins are also associated with liver cell damage.¹¹ The ALT, AST and ALP activity and serum TB level are largely used as the most common biochemical markers to evaluate liver injury. Administration of hepatotoxic agent caused significant elevation of enzyme level such as AST, ALT, ALP and bilirubin has been attributed to damaging the structural integrity of liver indicating development of hepatotoxicity.¹² The administration of plant extract has presented the decreased serum marker enzymes AST, ALT, ALP, total protein and total bilirubin level. This is in agreement with the commonly accepted view that serum levels of AST, ALT and ALP return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes. The hepatotoxic effects of CCl₄ are largely due to generation of free radicals. Drugs having antioxidant activity is effective in treating CCl₄ induced hepatotoxicity.¹³ Most of plants may have antioxidant activity due to presence of diterpenes, alkaloids and flavanoids because of number of scientific reports indicated that certain flavanoids, triterpenoids and steroids have protective effect on liver due to its antioxidant properties. The methanolic extract exhibit the excellent hepatoprotective properties as indicated by maximum prevention of increased serum biochemical parameters on CCl₄ induced toxicity. This plant extract has shown hepatoprotective activity may be through regulatory action on cellular permeability, stability and suppressing oxidative stress. This was supported by histopathological study.

CONCLUSION

Thus from the study, it can be concluded that the possible mechanism of hepatoprotective activity of methanolic leaves extract of *R. procumbens* may be due to the presence of flavonoids and diterpenes. The results of the present study suggest that MERP has hepatoprotective properties however

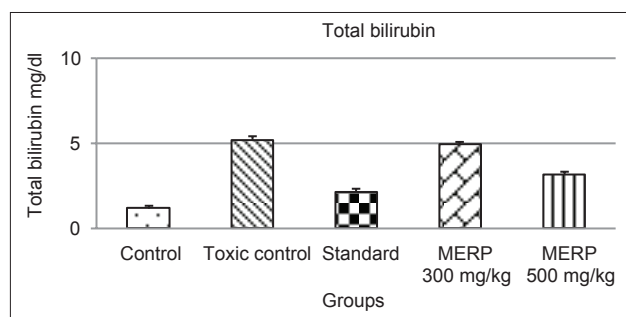


Figure 4: Effect of *Rostelluria procumbens* leaves extract on serum total bilirubin levels in carbon tetrachloride treated rats.

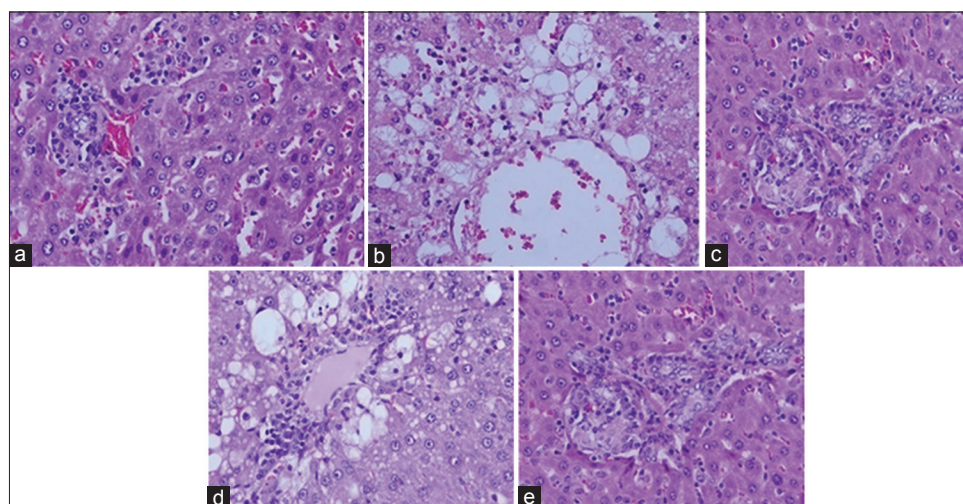


Figure 5: Effect of *Rostelluria procumbens* leaves extract on histology of carbon tetrachloride (CCl₄) treated rats, (a) section of the liver tissue of control animal, (b) section of liver tissue of animal treated with CCl₄, (c) section of the liver tissue of silymarin + CCl₄ treated animal, (d) section of liver tissue treated with MERP 250 mg/kg+ CCl₄, (e) section of the liver tissue treated with MERP 500 mg/kg + CCl₄.

studies are correctly being carried out to quantitatively and structurally determine phytochemical principles responsible for this action.

ACKNOWLEDGMENTS

We are thankful to TRR College of Pharmacy Management for funding and providing laboratory space and animal ethical committee approval for this research along with their valuable advice and information.

Funding: TRR College of Pharmacy

Conflict of interest: None declared

Ethical approval: TRR College of Pharmacy Animal Ethical Committee Approved (CPCSEA No. 1447/PO/a/11/CPCSEA)

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doi: 10.5455/2319-2003.ijbcp20141232

Cite this article as: Mamatha M, Manasa V, Vijusha M, Suthakaran R. Hepatoprotective activity of methanolic leaves extract of *Rostellularia procumbens* by using carbon tetrachloride intoxicated rats. *Int J Basic Clin Pharmacol* 2014;3:964-9.