International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 13-08-2021, Accepted: 27-08-2021, Published: 13-09-2021
Volume 6, Issue 5, 2021, Page No. 439-442

A comparative study between hepatoprotective and antioxidant activity by using leaf extract of *Momordica dioica roxb*

V Nandhinipriya[1], M Kumar[2], S Alexander[3], S Sangeetha[4]

[1] Lecturer, Department of Chemistry, Vinayaka Mission's College of Pharmacy (Autonomous) (Affiliated to Vinayaka Mission's Research Foundation, (DU), Salem, Tamil Nadu, India

[2] Professor and Head, Department of Chemistry, Vinayaka Mission's College of Pharmacy (Autonomous) (Affiliated to Vinayaka Mission's Research Foundation, (DU), Salem, Tamil Nadu, India

[3] Associate Professor, Department of Chemistry, Vinayaka Mission's College of Pharmacy (Autonomous) (Affiliated to Vinayaka Mission's Research Foundation, (DU), Salem, Tamil Nadu, India

[4] Assistant Professor, Department of Chemistry, Vinayaka Mission's College of Pharmacy (Autonomous) (Affiliated to Vinayaka Mission's Research Foundation, (DU), Salem, Tamil Nadu, India

Abstract
In this study, the ethanol extract and the water extract of Momordica charantia had hepatoprotective activity. The effects of the leaves on carbon tetrachloride (CCl4) -induced liver injury in rats were evaluated. The extract was administered orally once a day at a dose of 200 mg/kg. Serum glutamate oxaloacetate aminotransferase (AST) significantly increased, serum glutamate pyruvate aminotransferase (ALT), serum alkaline phosphatase (SALP) and total bilirubin Serum enzyme levels increased significantly by extract Return to normalization. Silymarin was used as standard reference material and showed significant hepatoprotective activity against carbon tetrachloride-induced contact toxicity in rats. The biochemical observation was supplemented by histopathological examination of liver slices in rats. The results of this study clearly indicate bitter gourd Roxb. The leaves have a strong hepatoprotective effect on the liver damage caused by carbon tetrachloride in rats. The results prove that ethanol extract is a more effective hepatoprotective agent. At the same time, the antioxidant and free radical scavenging activities in the body were also selected, which were positive for ethanol and water extracts. This study shows that the possible mechanism of this activity may be due to the antioxidant and free radical scavenging activities produced by the presence of flavonoids in the extract.

Keywords: *Momordica dioica roxb*, antioxidant activity, hepatoprotective activity, silymarin, DPPH

Introduction

Despite great advances in modern medicine, there are no effective drugs available to stimulate liver function and protect the liver from damage or help regenerate ingested liver cells (Chattopadhyay *et al*, 2003) [1]. The number of medicinal preparations recommended for the treatment of liver problems (Chatterjee *et al*, 2000) [2] and which are generally thought to have significant analgesic effects. Global efforts are being made to obtain scientific evidence for these traditionally reported herbal medicines. *Momordica dioica Roxb*. (MDR) is a climbing species and is called Kakora in Hindi. The root is used in headaches, painful urination, and as a remedy for jaundice. The leaves are an aphrodisiac, deworming, curing "tridhosa", fever, asthma, bronchitis, profuse cough, hemorrhoids, and "pitta" disease. Arhat fruit, laxative, cure "Vata", asthma, leprosy, bronchitis, fever, tumor, "tridosha", urinary, salivation, and heart problems heart (Kirtikar and Basu *et al*, 1999) [3]. This plant also has gastric-protective and ulcer-healing activities (Fernandopulle and Karunanyake *et al*, 1994; Fernandopulle and Ratnasooriya *et al*, 1996) [4, 5]. The fruits are reported for their hepatoprotective activity (Kushawa *et al*., 2005) [6]. According to ethnological claims, the leaves are used to treat jaundice and other liver diseases by folk tribes in the state of Tripura, India. To the best of our knowledge, there are no scientific reports supporting the hepatoprotective activity of MDR leaves. Therefore, to substantiate traditional claims, we evaluated the hepatoprotective effects of MDR leaves using CCl4intoxico mice.

Materials and methods

Animals

Albino Wister rats and mice of both sexes were used to study the crude extracts. The Tee Animal Ethics Commitment approved the project (918 / ac / 08 / CPCSEA). Animals were kept at 27 ° C, 44-56% relative humidity, and cycles of light and dark of 10 and 14 h, respectively, for 1 week before and during the experiment. The animals received standard food (Lipton, India) and the food was removed 18-24 h before the start of the experiment and given water. All experiments were performed in the morning according to current guidelines for the care of laboratory animals and ethical guidelines for investigating experimental pain in conscious animals (Zimmerman *et al*, 1983) [7].

Plant resources and preparation of raw medicinal extracts

MDR leaves were collected from the Ghabua district of Madhya Pradesh (MP), India, and identified in the

Government Department of Botany. P.G. College, Mandsaur (MP). Herb sample has been submitted to the College of Pharmacy Faculty (Support Form # -001/M). The leaves are shade-dried and degreased with petroleum ether. The degreasing material was extracted with 95% ethanol and then dried under a vacuum. Part of the powdered leaves is steeped in boiling water and the rest is soaked in water for 7 days and stirred occasionally. The decoction and decoction were filtered and dried under vacuum.

Phytochemical studies
All extracts were the subject of a phytochemical study (Khandelwal *et al*, 2005) [8].

Acute Toxicity Studies
Acute toxicity studies on ethanolic and aqueous extracts (decorated and soaked) of MDR leaves were performed in albino rats and rats. Animals were fasted overnight prior to the experiment and kept under standard conditions. All extracts were administered orally in gradually increasing doses and were found to be safe at doses up to 2000 mg/kg for all extracts.

Toxicity due to CCl4
Rats can be split into six divided groups (n = 6). Group I animals (controls) received a single dose of water (1 ml/kg, p. o.) per day for 5 days and received liquid paraffin (1 ml/kg, s.c.) on days 2 and 3. Group II (CCl4) received water (1 ml/kg BW, PO) once daily for 5 days and received CCl4: liquid paraffin (1: 1.2 ml/kg BW, sc) on day 2, and group III received the standard drug silymarin (50 mg/kg, PO) once daily for 5 days. The animals of the evaluation group (i.e Groups IV- group VI) were orally administered at a dose of about 200 mg/kg of aqueous (decoction, decoction) and ethanolic extract, respectively, once a day in aqueous suspension. Animals in groups III-VI were co-administered with CCl4: liquid paraffin (1: 1.2 ml/kg BW, s.c.) on days 2 and 3 after 30 minutes depending on silymarin and extract. The animals were given up after 24 hours of the last treatment. Blood was collected, allowed to clot, and serum was separated at 2500 rpm for 15 min and biochemical studies were performed. Livers were dissected and used for histopathology studies (Shanmugasundaram and Venkataraman, 2006) [9].

Biochemical determination
Biochemical parameters such as serum enzymes: aspartate aminotransferase (AST), glutamate pyruvate transaminase (ALT) (Reitman and Frankel *et al*, 1957) [10] serum alkaline phosphatase (SALP) (King *et al*, 1965) [11], and total bilirubin (Malloy and Evelyn *et al*, 1937) [12] were determined. determined by test kits (nostic Span Diag, Surah).

DPPH scavenging activity
The free radical scavenging activity of all extracts was measured in terms of hydrogen delivery capacity or radical scavenging capacity using stable radical DPPH (Blois *et al*, 1958) [26]. A solution of DPPH (0.1 mM) in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of solutions of all extracts in water at different concentrations (100-1000 µg. /ml). Absorbance was measured at 517 nm after thirty minutes. The minimum absorbance of the reaction mixture shows an increased free radical scavenging activity. Ascorbic acid is used as the standard drug.

Estimates of MDA, hydroperoxide, GSH, SOD, CAT
Division and dosing regimens in rats were followed in the same manner as mentioned in CCl4-induced toxicity. After 5 days of the administration, the animals were sacrificed by cervicectomy. Liver samples were dissected and immediately washed with cold saline to remove as much blood as possible. Liver homogenizers (5% w/v) were prepared in cold 50 mM potassium phosphate buffer (pH 7.4) using a Remi homogenizer. Intact cells and cell debris were removed by centrifugation at 1000 rpm for 10 rpm using a refrigerated Remi centrifuge. The supernatant was used to estimate the levels of GSH (Ellaman *et al*, 1959) [27], malondialdehyde (MDA) (Yagi and Rastogi *et al*, 1979) [13], hydroperoxide (Jaing *et al*., 1992) [14], superoxide dismutase (SOD) (Kakkar *et al*., 1972) [15], and catalase (Smna *et al*, 1972) [16].

Histopathology
Liver tissue was separated and fixed in 10% formalin, progressively dehydrated in ethanol (50-100%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with haematoxylin and eosin (H-E) dyes for microscopic observations including cell necrosis, fat transformation, hyaline degradation, bubble degeneration.

Statistical analysis
The data can be indicated as mean S.E.M. The variation between means had been determined by one-way ANOVA. The statistically significant value of $P < 0.05$ has been considered.

Results
Phytochemical study
An entire extract inflicts to the phytochemical studies indicates the presence of carbohydrates, alkaloids, proteins, phenolic compounds, amino acids, flavonoids, and glycosides.

Acute toxicity studies
Ethanolic and aqueous extracts did not show any signs and symptoms of toxicity and mortality up to 2000 mg/kg dose.

Effects of extracts on ALP, ALT, AST, and total bilirubin
The results of the hepatoprotective effect of extracts on CCl4-intoxicated rats are shown. In the CCl4 intoxication group (II), serum AST, ALT, ALP, and total bilirubin increased to 466.5 U/L, 252.51 U/L, 285.35 IU/L, and 5.19 mg/dL, respectively, but these values were 99.67. In the control group (I), U/L, 50.02 U/L, 127.43 IU/L, and 1.19 0.131 mg/dL, respectively. The elevated levels of serum AST, ALT, ALP, and total bilirubin were significantly reduced in the animal groups treated with various extracts. Analysis with ethanolic extract results with raised significant activity ($P<0.001$) with inhibition maximum. Therefore, the ethanolic extract-analysed group was effective compared to the other extracts but it is not much superior to the silymarin. Compared to all, aqueous extracts decoction results increased significantly ($P<0.01$) and inhibition better.

DPPH-scavenging activity

It shows that the free radical concentration of DPPH was significantly reduced due to the scavenging ability of the extract.

The results show that the ethanol extract has a better purifying activity and increases as the concentration increases. The IC50 of ascorbic acid was found to be 132.12 ± 7.23 µg / ml.

Effects of extracts on hydroperoxides, MDA, SOD, GSH, CAT levels

Results of the study clearly revealed an increase in the levels of MDA and hydroperoxides in CCl4- intoxicated rats compare to the control group. Treatment with extracts significantly prevented this rise in levels. GSH, SOD, and CAT content have significantly increased in extract-treated groups whereas the CCl4-intoxicated group has shown a significant decrease in levels compared to the control group. An ethanol extract showed the greatest protection, followed by maceration and decoction.

Histopathological observations

The histology of liver sections of control animals (group I) showed that normal liver cells had well-preserved cytoplasm, prominent nuclei, nucleoli, and visible central veins. Liver sections of carbon tetrachloride poisoned rats showed extensive fat changes, necrosis, balloon degeneration, extensive lymphocyte infiltration, and loss of cell boundaries. The histological structure of rat liver slices treated with water and ethanol extracts showed a more or less normal lobular pattern, accompanied by slight changes in fat, necrosis, and lymphocyte infiltration, almost equivalent to the control group and the silymarin-treated group.

Conclusion

In this study, CCl4-induced hepatotoxicity in the rat model was used to evaluate the hepatoprotective activity of water and ethanol extracts from MDR leave and find the extract with the best therapeutic effect. Try to find the correlation between antioxidant and hepatoprotective activity. This research also provides some scientific basis for the influence of the extraction solvent and the extraction method.

CCl4 is widely used to study the hepatoprotective activity of various experimental animals (Bhathal *et al*., 1983) [17]. An important defense mechanism involves antioxidant enzymes, including SOD, catalase, and glutathione peroxidase (GPx), which convert reactive oxygen molecules into non-toxic compounds.

When the double bond in the structure of unsaturated fatty acids loses a hydrogen atom to form free radicals, lipid peroxidation will accelerate. Scavenging free radicals is one of the main antioxidant mechanisms that inhibit the lipid peroxidation chain reaction. The free radical scavenging activity of crude drug extracts was evaluated by DPPH assay. It is well known that DPPH can extract unstable hydrogen (Constantin *et al*., 1990; Matsubara *et al*., 1991) [18, 19]. The uptake of DPPH free radicals is related to the inhibition of lipid peroxidation (Ratty *et al*., 1988; Rekka and Kourounakis, 1991) [20, 21]. At the same time, compare the hepatoprotective effects of MDR leaf extracts with those treated with silymarin, which is the active ingredient in Silybum fruit (Silybum). mar ianum, Compositae). Test the antioxidant activity in the body by estimating the levels of MDA, hydroperoxide, GSH, SOD, and CAT.

Liver damage is assessed by biochemical studies (AST, ALT, ALP, and total bilirubin) and histopathological examinations. CC14 produces experimental damage histologically similar to viral hepatitis (James and Pickering, 1976) [22]. Toxicity begins with changes in the endoplasmic reticulum, leading to the loss of metabolic enzymes located in the intracellular structure (Recnagal, 1983) [23]. Produces toxic free radical metabolite CC13, which further reacts with oxygen to generate peroxytrichloromethyl free radicals. 2E1 Enzyme Cytochrome P450 is responsible for these conversions. Free radicals bind covalently to macromolecules and cause peroxidative degradation of the lipid membrane of adipose tissue. From this point of view, the extract reduced AST and ALT levels, indicating the stability of the plasma membrane and the repair of liver tissue damage caused by CC14. This effect is consistent with the generally accepted view that serum transaminase levels return to normal with the healing of liver parenchyma and the regeneration of liver cells (Thabrew *et al*., 1987) [24]. Alkaline phosphate is the prototype for these enzymes, reflecting pathological changes in bile flow (Ploa and Hewitt, 1989) [25]. CCl4 induces an increase in the activity of this enzyme in the serum that is consistent with the high level of serum bilirubin. The ethanol extract induces and inhibits increased SALP activity while reducing elevated bilirubin, indicating that the extract can stabilize rat liver biliary dysfunction during carbon tetrachloride liver injury. Therefore, the application of ethanol and water leaf extracts revealed the hepatoprotective activity of MDR leaves against the toxic effects of CCl4, which was also supported by histological studies. As the preliminary phytochemical analysis of the extract showed the presence of flavonoids and phenolic compounds, these compounds are known for their antioxidant and hepatoprotective activities. Pathological changes caused by CCl3 free radicals play a beneficial role. The elimination of free radicals is one of the main antioxidant mechanisms that inhibit the chain reaction of lipid peroxidation. A significant decrease in the levels of MDA and hydroperoxide in the group treated with the extract indicates a decrease in lipid peroxidation. The simultaneous significant increase in the contents of GSH, SOD, and CAT in the liver indicates the antioxidant activity of MDR leaf extract and silymarin. Therefore, it can be concluded that the possible mechanism of the hepatoprotective activity of multidrug-resistant leaves may be due to their antioxidant and free radical scavenging activities, which may be due to the presence of flavonoids and phenolic compounds in the extract. However, more studies are needed to confirm the involvement of cytochrome P450 enzyme inhibition. It was also concluded that the ethanol extract that inhibited free radicals to the greatest extent was the most effective extract among the tested extracts. The decoction has better activity than the macerator. More research is underway to better understand the mechanism of action and its anti-hepatitis activity.

Reference

1. Chattopadhyay RR. Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract. Part II. Journal of Ethnopharmacology,2003:89:217-219.

2. Chatterjee TK. Medicinal Plants with Hepatoprotective Properties in Herbal Opinions, vol. III. Books and Allied (P) Ltd., Calcutta, 2000, 135.
3. Kirtikar K, Basu BD. Indian Medicinal Plants, vol. II. International Book Distributors, Dehradun, 1999,1 129-1135.
4. Fernandopulle BMR, Karunanyake EH. Oral hypoglycemic effect of, 1994.
5. Fernandopulle BMR, Ratnasooriya WD. Evaluation of two cucurbits (Genus: *Momordica*) for gastroprotective and ulcer healing activity in rats. Medical Science Research,1996:24:85-88.
6. Kushawa SK, Jain A, Jain A, Gupta VB, Patel JR, Dubey PK. Hepatoprotective activity of fruits of *Mormordicadioica* Roxb. Plant Archive,2005:5:613-616.
7. Zimmerman M. Ethical guidelines for investigation of experimental pain in conscious animal. Pain,1983:16:109-110.
8. Khandelwal KR. Practical Pharmacognosy, XIV ed. Nirali Prakashan, Pune, 2005, 149-153.
9. Shanmugasundaram P, Venkataraman S. Heapatoprotective and antiox- idant activity of *Hygrophila auriculata* (K. Schum) Heine Acanthaceae root extract. Journal of Ethnopharmacology,2006:104:124-128.
10. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamate oxaloacetate transaminase. American Journal of Clinical Pathology,1957:28:53-56.
11. King J. The hydrolases-acid and alkaline phosphatases. In: Prac- tical Clinical Enzymology. Nostrand Company Limited, London, 1965, 191-208.
12. Malloy HT, Evelyn KA. The determination of bilirubin with the photometric colourimeter. Journal of Biological Chemistry,1937:119:481-490.
13. Yagi K, Rastogi R. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry,1979:95:351-358.
14. Jaing ZY, Hunt JV, Wolff PS. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroxide in low density lipoprotein. Analytical Biochemistry,1992:202:384-389.
15. Kakkar P, Das B, Visvanathan PN. A modified spectrophotomet- ric assay of superoxide dismutase. Indian Journal of Biochemistry,1972:197:588-590.
16. Smna KA. Colourimetric assay of catalase. Analytical Biochemistry,1972:47:389-394
17. Bhathal PS, Rose NR, Mackay IR, Whittingham S. Strain differ- ences in mice in carbon tetrachloride-induced liver injury. British Journal of Experimental Pathology,1983:64:524-533.
18. Constantin M, Bromont C, Fickat R, Massingham R. Studies on the activity of Bepridil as a scavenger of free radicals. Bio-chemical Pharma- cology,1990:40:1615-1622.
19. Matsubara N, Fuchimoto S, Iwagaki H, Nonaka Y, Kimura T, Kashino H *et al*. The possible involvement of free radical scavenging properties in the action of cytokines. Research Communications in Chemical Pathology and Pharmacology,1991:71:239-242.
20. Ratty AK, Sunamoto J, Das NP. Interaction of flavonoids with 1,1-diphenyl-2-picrylhydrazyl free radical, liposomal membranes and soyabean lipoxygenase 1. Biochemical Pharmacology,1988:37:989-995.
21. Rekka E, Kourounakis PN. Effect of hydroxyethyl rutenosides and related compounds on lipid peroxidation and free radical scavenging activity-some structural aspects. Journal of Pharmacy and Pharmacology,1991:43:486-491.
22. James GWL, Pickering RW. The protective effect of a novel compound RU-18492 on galactosamine induced hepaptotoxicity in rats. Drug Research,1976:26:2197-2199.
23. Recnagal RO. A new direction in the study of carbon tetrachloride hepatotoxicity. Life Sciences,1983:33:401-408.
24. Thabrew MI, Joice PDTM, Rajatissa WA. Comparative study of efficacy of *Paetta indica* and *Osbeckia octandra* in the treatment of liver dysfunction. Planta Medica,1987:53:239-241.
25. Ploa GL, Hewitt WR. In: Wallace Hyes, A. (Ed.), Principle and Meth- ods of Toxicology. Raven Press, New York,1989:2:399.
26. Blois MS. Antioxidant determination by the use of a stable free radical, 1958.
27. Ellaman GL. Tissue sulfhdryl group. Archives of Biochemistry and Biophysics,1959:82:70-72.