Antioxidant and Hepatoprotective Activity of the Aqueous Extract of Myrtus Communis (Myrtle) Linn. Leaves

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Summary

The aqueous extract of Myrtus communis leaves was screened for hepatoprotective activity in paracetamol induced hepatotoxicity in albino rats. The degree of protection was measured by estimating biochemical parameters like serum glutamate pyruvate transaminase, Serum glutamate oxaloacetate transaminase, serum alkaline phosphatase , total protein and level of serum bilirubin (both total and direct). Hepatoprotective activity of aqueous extract at a dose of 200 mg/kg and 400 mg/kg body weight, p.o., was compared with Silymarin (100mg/kg, p.o.) treated animals. Myrtus communis leaves (200 and 400 mg/kg) exhibited significant reduction in serum hepatic enzymes when compared to rats treated with paracetamol alone. Furthermore, histopathological studies were also done to support the study.

Keywords: Paracetamol, hepatoprotective activity, Silymarin, Myrtus communis

Introduction

Herbs play a major role in the management of various liver disorders along with other system associated diseases. Liver is a key organ regulating homeostasis within the body by various functions. Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Hepatotoxicity is one of very common aliment resulting into serious debilities ranging from severe metabolic disorders to even mortality1. Plant derived natural products such as flavonoids, terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity.1-3.

Among the medicinal plants, *Myrtus communis* (Family – Myrtaceae) (known as Myrtle) is a useful Indian medicinal plant which has been credited with therapeutic properties to treat several diseases. The plant has been reported to contain Isolation and structure elucidation of two acylphoroglucinols A and B. Limonene, linalol, α-pinenine, cineol, p-cymol, camphene, β-pinene, traces of car-3-ene found in leaf essential oil. The leaves also contain tannins and polyphenolics.4,5

The present study was undertaken to study the possible hepatoprotective role of aqueous extract of leaves of *Myrtus communis*. Paracetamol (acetaminophen) is a widely used antipyretic and analgesic which produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates 6, 7. However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites

when a part of paracetamol is activated by hepatic cytochrome P-45012 to a highly reactive

metabolite N-acetyl-P-benzoquinone imine (NAPQI) 13. NAPQI is initially detoxified by

conjugation with reduced glutathione (GSH) to form mercapturic acid 14. However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or SH group of protein and alters the homeostasis of calcium after depleting GSH. Silymarin is marketed as one of the standard hepatoprotective herbal formulation.

Materials and methods

Plant material

The Myrtus communis leaves were collected from garden in East Godavari dist and authenticated by the taxonomist of Department of Botany, Calicut University. The voucher specimen was deposited in the herbarium of university for future reference.

Preparation of extracts

The material was air dried under shade, powdered mechanically and stored in airtight containers. About 1000 gm of the powdered material was subjected to cold maceration for 7 days with continuous stirring. The extract was filtered and the filtrate was concentrated at reduced pressure by Rotary Vacuum Evaporator.

Experimental animals

Albino rats of either sex (150-200 gm) were used for the study. The animals were procured and housed in the animal house maintained under standard hygienic conditions, at 25 ± 1o C, humidity (60 ± 10%) with 12 hour day and night cycle, with food and water *ad libitum*. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC) of Bharathi College of pharmacy, Bharathinagara, Mandya. Studies were performed in accordance with the CPCSEA guidelines.

Hepatoprotective activity

The LD50 is >1g/kg. No toxic effects or mortality were observed with doses ranging from 50mg/kg to 1g/kg for four weeks. Acute and sub acute (15-30 days administration) toxicity studies did not detect any changes in vital organ function tests.[19] Hence hepatoprotective activity of Aqueous extracts of *Myrtus communis* was studied by following methods.

Paracetamol induced-hepatotoxicity:

Group A - Normal control

Group B - Toxicant (paracetamol 500mg/kg, p.o.)

Group C - Served as Standard (Silymarin 100 mg/kg, p.o.)

Group D - Aqueous extract of Myrtus communis leaves (200mg/kg, p.o.)

Group E - Aqueous extract of Myrtus communis leaves (400mg/kg, p.o.)

Experimental procedure

Albino rats of either sex weighing between 150-200 g were divided into five groups of six rats each. Group A was maintained as normal control, which was given distilled water only. Group B received paracetamol 500 mg/kg body wt by p.o at every 72 hours for 10 Days. Group C animals were treated with Silymarin (100 mg/kg p.o) which served as standard. Groups D and E animals were treated with two different doses of Aqueous extract of *Myrtus communis leaves* (medium, high) respectively Group C, D and E were in toxicated with paracetamol (500 gm/kg) 1 hr before the administration of Silymarin or extract for 10 days. The animals were then anesthetized using anesthetic ether, and blood collected by retro orbital puncture and biochemical parameters like ALT, AST, ALP, Total Bilirubin, were estimated. The animals were sacrificed by overdose of ether and autopsied. Livers from all animals were removed, washed with ice-cold saline, weighed. Small piece of liver tissue was collected and preserved in 10% formalin solution for histopathological studies. Livers of some animals were homogenized with ice-chilled 10% KCl soln and centrifuged at 2000 rpm for 10 minutes. Then the supernatant liquid was collected and the antioxidant parameters like Catalase and Super oxide Dismutase were estimated.

Statistical analysis

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as significant.

Results

Biochemical Parameters

The animals treated with Paracetamol exhibited a significant (P,0.01) rise in SGOT, SGPT, ALP and bilirubin levels when compared to the control group. This was significantly (P, 0.01) reduced after treatment with AEMC-first, which was almost similar to that of Silymarin

Lipid Peroxidation

The liver MDA, which is an index of tissue lipid peroxidation, was found to be significantly (P, 0.01) higher in the Paracetamol-treated group than measured in the control group. Treatment with AEMC first decreased the elevated MDA levels. The MDA level for Silymarin was also found to be significantly decreased.

Total Protein

Total protein level was significantly (P, 0.01) reduced in the Paracetamol-treated group when compared to the control and was significantly elevated in the AEMC-first-treated groups. This was comparable to that of Silymarin-treated group

Antioxidant Enzymes and Glutathione Levels

The levels of antioxidant enzymes such as CAT and SOD and LPO were decreased significantly (P, 0.05) after Paracetamol treatment and was significantly (*P*, 0.01) elevated in AEMC -first-treated group. This was comparable with that of Silymarin-treated group

	Normal control	Toxicant control	Standard	AEMC 200mg/kg	AEMC 400mg/kg
WET LIVER WEIGHT	2.15 ± 0.95	4.50 ± 0.096	2.6 ± 0.066***	3.083±0.070*	2.61±0.116***
SGPT	25.42±0.17	108.31±0.23	48.91±0.082***	83.52±0.20**	69.27±0.208**
SGOT	30.19±0.605	150.94 ±1.661	78.87±0.717**	115.51±0.88*	94.64±0.885**
ALP	25.86±0.914	170.66±1.909	32.76±0.305***	80.17±0.56*	56.17±0.577**
TOTEL BILIRUBIN	0.32±0.018	1.80±0.057	0.81±0.026***	1.6±0.057*	1.215±0.053**
CAT	86.38±0.60	24.83±0.660	74.16±0.94***	32.16±0.60*	46.5±0.76**
SOD	10.5±0.76	3.75±0.156	8.30±0.12***	5.4±0.09*	6.73±0.09**
LPO	3.43±0.05	8.31±0.08	5.33±0.08***	7.7±0.09**	6.7±0.09**

Values are expressed in mean ± SEM (n=6) one way ANOVA followed by Tukey-Kramer's test. Where, * represents significant at p<0.05, ** represents highly significant at p< 0.01, *** represents very significant at p<0.001

Histopathological examination of the liver section of the rats treated with toxicant showed intense Effaced architecture, Apoptotic hepatocytes and Congested central veins. The rats treated with Silymarin and extract along with toxicant showed sign of protection against these toxicants to considerable extent as evident from formation of normal hepatic cards and absence of Effaced architecture, Apoptotic hepatocytes and Congested central veins.

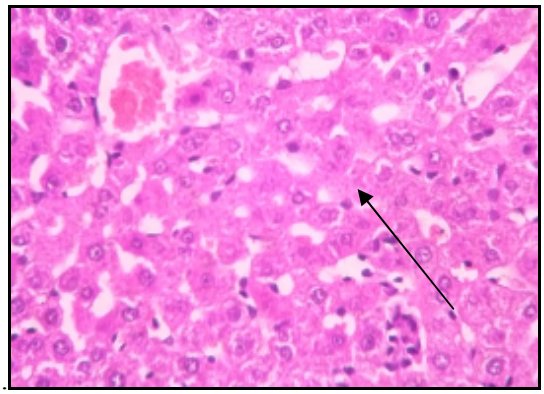

Fig.1.Liver tissues of Normal control.

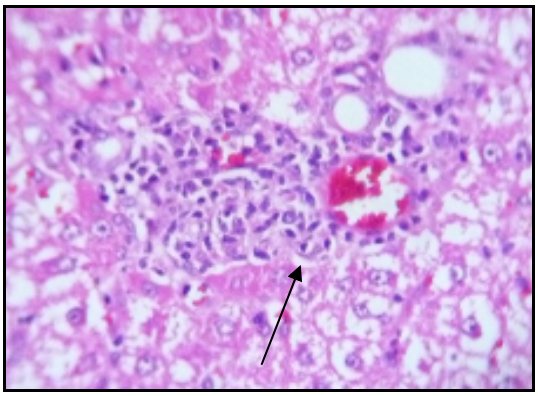

Fig.2.Liver tissue of Paracetamol treated groups

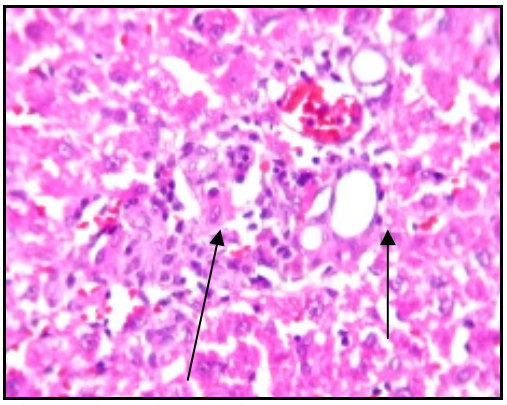

Fig.3. Liver tissue of Aqueous extract

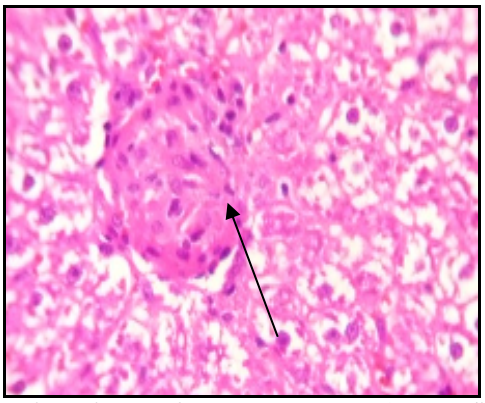

Fig.4.Liver tissue of Aqueous extract (400mg/kg)

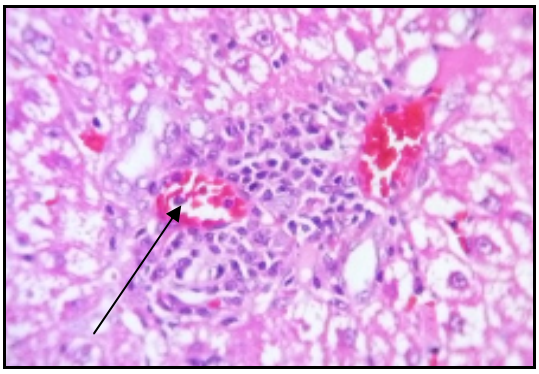

Fig 5.Liver tissue of Silymarin + Paracetamol treated groups (200mg/kg)

Discussion

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases. There is a growing interest in the pharmacological evaluation of various plants used in Ayurvedic system of medicine. [22] In the assessment of liver damage by Paracetamol, the determination of enzyme levels was used. Serum SGPT, SGOT, ALP and bilirubin are the most sensitive markers used in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage. In this study, an increase in the activities of SGPT, SGOT, ALP and bilirubin in serum evidenced the Paracetamol-induced hepatocellular damage. [23-26]

The reduction of Paracetamol-induced elevated plasma activities of these enzyme levels in animals treated with the formulation showed their ability to restore the normal functional status of the damaged liver.[25,26] The determination of malondialdehyde (MDA) level is one of the most commonly used methods for monitoring lipid peroxidation.[24]

The result suggests that there was a dramatic increase in lipid peroxidation after Paracetamol treatment and it was inhibited by the treatment with the extraction revealing that it exhibits potent hepatoprotective activity. Measurement of protein concentration was mainly used to calculate the level of purity of a protein. Maximum doses of Paracetamol cause depletion of total proteins indicating tissue damage which was also evidenced in this study.

Treatment with Paracetamol significantly decreased GSH, CAT and SOD stores indicating that they were used for the detoxification of toxic metabolites of the drug. The extraction restored the antioxidant enzyme levels significantly and reduced the Paracetamol-induced oxidative injury, thus proving its antioxidant potential. [19]
The histopathological examination of the liver of the control group showed normal hepatocytes with portal triad [Figure 1]. The liver section of Paracetamol-treated rats showed typical centribular hepatocytic steatosis (both macrovesicular and microvesicular) and necrosis, limiting plate necrosis, apoptosis especially in the periportal hepatocytes and portal triaditis [Figure 2].This could be due to the formation of highly reactive free radicals because of oxidative stress caused by Paracetamol. Simultaneous administration of formulation along with Paracetamol prevented these effects [Figures 3 and 4]. Thus, histopathological studies revealed that concurrent administration of Paracetamol with the extraction exhibited protection of liver cells, which further confirmed the above results.

Conclusions

The results of this study clearly demonstrated that the formulation exhibited potent hepatoprotective activity against Paracetamol-induced hepatic damage in rats. This may be due to their antioxidative and free radical scavenging properties. Further studies are needed to isolate and purify the active principles involved in the individual plants of the formulation for confirming the hepatoprotective efficacy.

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References

1. DeFeuids FV, Papadopoulos V, Drieu K. *Ginko biloba* extracts and cancer a research area in its infancy. Fundam Clin Pharamacol 2003; 17:405-17.
2. Banskota AH, Tezuka Y, Adnyana IK, Xiong Q, Hase K, Tran KQ, *et al* Hepatoprotective effect of *Commbretum quadrangulare* and its constituents. Biol Pharm Bull 2000; 23:456-60.
3. Takeoka GR, Dao LT. Antioxidant constituent of almond (*Prunusdulcis* (Mill.) D.A.Webb) hulls. J Agri Food Chem 2003;51:496-501
4. El-Sissi HI, Ansary MA. Tannis and polyphenolics of the leaves of *Myrtus communis*. Planta Med.1967; 15:41-51.42
5. Dogan A. Investigations *Myrtus communis* L. plant's volatile oil yield their physical-chemical properties and their compositions. Turkish: Ankara University, Agricultural Faculty Press; 1978. p. 678.43
6. Gupta AA, Siddiqui IR, Singh J. A new anthraquinone glycoside from the roots of *Plumbagozeylanica.* Indian J Chem 2000; 398:796- 8.
7. Gupta MM, Verma RK, Uniyal GC, Jain SP. Determination of Plumbagin by normal phase high performance liquid chromatography. J Chromat 1993;637:209-12
8. Ghosh MN. Fundamentals of experimental pharmacology. 3rd ed. Kolkata: Hilton and Co; 2005. p. 192.
9. Samudram P, Rajeshwari H, Vasuki R, Geetha A, Sathiamoorthy P. Hepatoprotective activity of Bi-herbal ethanolic extract on Paracetamol induced hepatic damage in rats. Afr J Biochem Res 2008; 2:61-5.
10. .GPT/ALAT UV Kinetic Test [package insert]. Goa: Coral Pvt. Ltd; 2010
11. GOT/ASAT UV Kinetic Test [package insert]. Alkaline Phosphatase P-NPP Method [package insert]. Goa: Coral Pvt. Ltd; 2010
12. Bilirubin Total and Direct [package insert]. Goa: Coral Pvt. Ltd; 2010
13. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochem 1979; 95:351-8.

14. Naik RS, Mujumdar AM, Ghaskadbi S. Protection of liver cells from ethanol toxicity by curcumin in liver slice culture *in vitro*. J Ethnopharmacol 2004; 95:31-7.
15. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ. Protein measurement with the Folin Phenol reagent. J Biol Chem 1951; 193:265-75.
16. Pari L, Latha M. Protective role of *Scoparia dulcis* plant extract on brain antioxidant status and lipd peroxidation in STZ diabetic male *Wistar* rats. BMC Compliment Altern Med 2004; 4:6882-4.
17. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys1959; 82:70-7.
18. Sinha AK. Colorimetric assay of catalase. Analytical Biochem 1972; 47:389-94.
19. Budhwar R, Kumar S. Prevention of chromate induced oxidative stress by alpha-lipoic acid. Indian J Exp Biol 2005; 43:531-5.
20. Misra HP, Fridovich I. The role of superoxide anion in the auto oxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; 247:3170-5.
21. Galigher AE and Kozloff EN; *Essential Practical Micro technique*, 2nd ed, Lea and Febiger, Philadelphia1971; p.77.
22. Malhotra S, Singh AP. Hepatoprotective natural products. Available from: http://www.siv.edu/ebl/leaflets/hepato.htm. [Cited on 2006 Nov 11].
23. Kind PRN, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Pathol 1954; 7:322-6.
24. Drotman RB, Lawhorn GT. Serum enzymes as indicators of chemically induced liver damage. Drug Chem Toxicol 1978; 1:163-71.
25. Reitman's S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957; 28:56-63.
26. Pari L, Amali DR. Protective role of tetrahydrocurcumin, an active principle of turmeric on chloroquine-induced hepatotoxicity in rats. J Pharm Pharmaceut Sci 2005; 8:115-23.