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HEPATOPROTECTIVE OF THE METHANOLIC EXTRACT OF *MYRTUS COMMUNIS*.LINN AGAINST CARBON TETRACHLORIDE (CCl₄) INDUCED HEPATOTOXICITY ON WISTAR RATS

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ABSTRACT

The methanolic extract of Myrtus communis leaves was screened for hepatoprotective activity in carbon tetrachloride 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 methanolic 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 carbon tetrachloride alone. Furthermore, histopathological studies were also done to support the study.

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Key Words

Carbon tetrachloride,
hepatoprotective activity,
Silymarin, Myrtus communis

INTRODUCTION

Myrtus communis Linn.(Family;Myrtaceae)commonly known as myrtle. It is a perennial plant Myrtle is a evergreen shrub , People living in Mediterranean region have consumed myrtle fruits, called as ‘hambeles’, ‘mersin’ or ‘murt’ in Turkish, as food, and used them for some medicinal purposes.^[1]

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^[6]. The leaves also contain tannins and polyphenolics^[2, 3].

In the present study, the hepatoprotective effects of methanolic extract (flavonoid rich fraction) are investigated in a scientific manner to validate its use as alternative and complimentary herbal medicine.

MATERIALS AND METHODS

Plant Material

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 Extract

The air-dried and coarsely powdered leaves of plant (1 kg) were Soxhlet Extracted with methanol for 72 h and the methanolic extract was concentrated on a water bath and dried under reduced pressure to get a dark brown mass (75 g).

Animals

Adult Wistar male albino rats weighing between 150 and 200 g were used for the study. They were kept under standard laboratory conditions and were fed with commercial rat pellets and drinking water *ad libitum*. The animals were housed in polypropylene cages. Ethical committee in accordance with animal experimentation and care has approved all animal procedures

Drugs and Chemicals

Carbon tetrachloride - SD Fine Chemicals, Mumbai
Silymarin - Microlabs, Bangalore
Reduced glutathione - HiMedia Laboratories Pvt.Ltd,

Mumbai

Chem. Kit for SGOT estimation - (Coral clinical systems, Verna Goa, India)

Chem. Kit for SGPT estimation - (Coral clinical systems, Verna Goa, India)

Chem. Kit for SALP estimation - (Coral clinical systems, Verna Goa, India)

All drugs and chemicals were purchased commercially and were of analytical grade

Experimental Design

Induction of experimental hepatotoxicity

Hepatotoxicity was induced by injecting CCl_4 intraperitoneal at a dose of 1 ml/kg body weight for 7 consecutive days.

Evaluation of hepatoprotective activity

Animals were divided into five groups, consisting of six animals each. The rat dose was calculated on the basis of the surface area ratio.^[4]

Group A - Normal control (Normal saline 10 ml/kg, p.o)

Group B - Toxicant (CCl_4 (1 ml/kg, i.p)

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

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

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

All the groups were treated for 7 consecutive days.^[5] At the end of this period, animals were kept overnight fasting and were sacrificed. Blood samples were withdrawn, serum separated and estimated for biochemical parameters. Liver tissues were removed for the determination of antioxidant enzyme levels and histopathological examinations.

Measurement of Biochemical Parameters

Blood samples were collected from retro-orbital plexus under ether anaesthesia and the serum was used for the assay of marker enzymes namely SGPT, SGOT, ALP and bilirubin. The enzyme levels were assayed using standard kits obtained from Coral clinical systems, Verna Goa, India^[6-9] The liver homogenate was prepared and the clear supernatant was used for the estimation of lipid peroxidation (MDA),^[10,11] total protein,^[12] reduced glutathione (GSH)^[13,14] and

antioxidant enzymes viz. Catalase (CAT) ^[15, 16] and superoxide dismutase (SOD) ^[17] level.

Histopathological Examination

A portion of liver tissue from each group was preserved in a 10% formaldehyde solution for histopathological studies. Haematoxylin and eosin were used for staining and later the microscopic slides of the liver cells were photographed at a magnification of 3100.

Statistical Analysis

Values were represented as mean \pm SEM. Data were analysed by one-way analysis of variance (ANOVA) followed by Tukey-kramer's test using statistical package for social sciences (SPSS) version Demo. P, 0.05 was considered significant.

RESULTS

Biochemical Parameters

The animals treated with CCl₄ 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 MEMC-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 CCl₄-treated group than measured in the control group. Treatment with MEMC first decreased the elevated MDA levels. The MDA level for Silymarin was also found to be significantly decreased.

Antioxidant Enzymes and Glutathione Levels

The levels of antioxidant enzymes such as CAT and SOD and LPO were decreased significantly (P, 0.05) after CCl₄ treatment and was significantly (P, 0.01) elevated in MEMC -first-treated group. This was comparable with that of Silymarin-treated group.

Histopathology

The histopathological examination showed that treatment with CCl₄ caused typical centrilobular hepatocytic steatosis (both macrovesicular and microvesicular) and necrosis, limiting plate necrosis, apoptosis, especially in the periportal hepatocytes and portal triaditis as compared with control liver. Liver tissues exposed to MEMC -first and Silymarin were

almost similar to the control in histology, size and staining properties and showed only mild congestion. In the formulation-treated group, there was reduction in inflammation and it significantly prevented the degeneration of hepatocytes. Thus, histological examination clearly demonstrated the protection of liver against CCl₄ cytotoxicity.

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. ^[18] In the assessment of liver damage by CCl₄, 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 CCl₄-induced hepatocellular damage. ^[19-22]

The reduction of CCl₄-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. ^[21,22] The determination of malondialdehyde (MDA) level is one of the most commonly used methods for monitoring lipid peroxidation. ^[20]

The result suggests that there was a dramatic increase in lipid peroxidation after CCl₄ 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 CCl₄ cause depletion of total proteins indicating tissue damage which was also evidenced in this study.

Treatment with CCl₄ 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 CCl₄-induced oxidative

injury, thus proving its antioxidant potential. ^[11]

The histopathological examination of the liver of the control group showed normal hepatocytes with portal triad [Figure 1]. The liver section of CCl₄-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 CCl₄. Simultaneous administration of formulation along with CCl₄ prevented these effects [Figures 3 and 4]. Thus, histopathological studies revealed that concurrent administration of CCl₄ with the extraction exhibited protection of liver cells, which further confirmed the above results.

	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**
TOTAL 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 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, and *** represents very significant at p<0.001. \

CONCLUSION

The results of this study clearly demonstrated that the formulation exhibited potent hepatoprotective activity against CCl₄-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.

Histopathological studies of the liver in CCl₄ induced hepatotoxicity

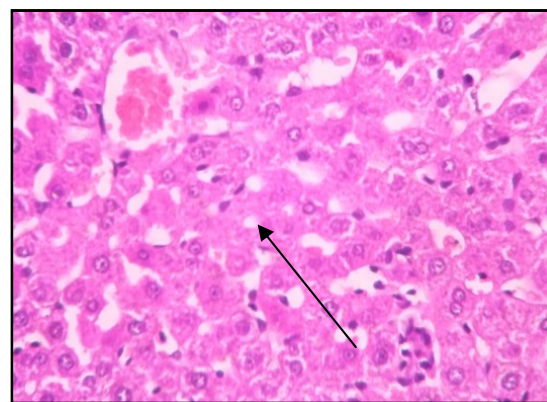


Fig 1 Liver tissues of Normal control groups

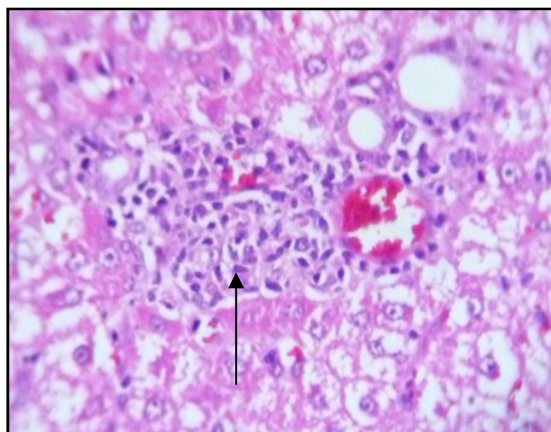


Fig 2 Liver tissue of CCl₄ treated groups

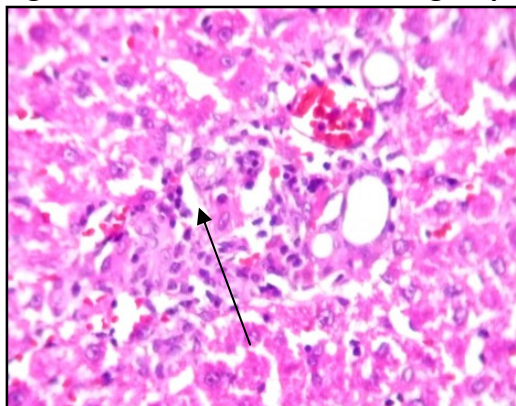


Fig 3 Liver tissue of Methanolic extract (200mg/kg) + CCl₄ treated groups

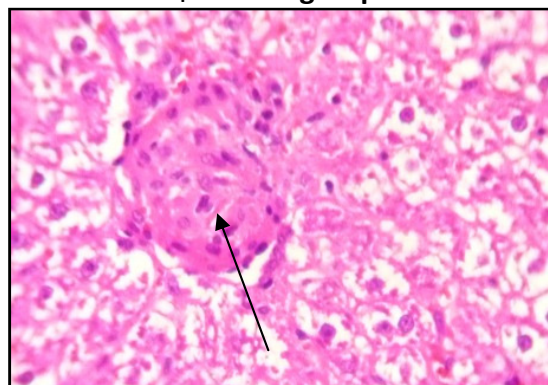


Fig 4 Liver tissue of Methanolic extract (400mg/kg) + CCl₄ treated groups

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