Hepatoprotective Activity of Methanolic Extract of Leaves of *Marsilea minuta* Linn Against CCl$_4$ Induced Hepatic Damage in Rats

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Abstract: In the present study *Marsilea minuta* (Marsileaceae) was evaluated for hepatoprotective, antihepatotoxic activities and antioxidative potential. The activities of the methanol extract of *Marsilea minuta* (MMME) whole plant were determined using carbon tetrachloride (Ccl$_4$) induced liver injury in rats. Male Wister albino rats were used in the study and showed no mortality or symptoms of toxicity upto a dose level of 2000 mg/kg b.w.p.o. for 72 hrs. Hence three test doses 100, 200, 400 mg/kg b.w.p.o. were taken in the study. Silibinin 50 mg/kg b.w.p.o was used as reference standard drug. Liver damage was achieved by injecting CCl$_4$ in olive oil (1:1) 1 ml/kg. It significantly(P<0.01) elevated the levels of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin (TP), direct bilirubin (DB) and lowered the levels of total protein (TP) and albumin (ALB). MMME at dose 200 mg/kg exhibited a significant hepatoprotective and antihepatotoxic activities by significantly (P<0.01) reversing the effects of CCl$_4$ induced hepatotoxicity. These biochemical observations were substantiated by histopathological examination of liver sections. Further, anti-oxidant activity invitro by DPPH method have been investigated to elucidate the possible mechanism of its hepatoprotective properties. The MMME exhibited a significant (P<0.01) anti-oxidant activity. Hence the plant was scientifically investigated for its traditional claim in the treatment of hepatitis.

Key words: *Marsilea minuta* · Hepatoprotective activity · Anti-hepatotoxic activity · Carbon tetrachloride · DPPH

INTRODUCTION

The liver plays an astonishing array of vital functions in the maintenance and performance of the body [1]. The burden of metabolism and exposure to various toxic substances make liver vulnerable to a variety of disorders.

Among the various disorders, Chronic hepatic diseases stand as one of the foremost health troubles worldwide, with liver cirrhosis and drug induced liver injury [2]. Herbal medicines are believed to be much safer and proved elixir in the treatment on various ailments [3]. Though the medicinal plants which have been used in the treatment of liver disorders many of them are yet to be proved scientifically. Therefore, there is a need for identification of such plants for scientific pharmacological investigation. In the present study, the plant *Marsilea minuta* was evaluated for its possible hepatoprotective and antihepatotoxic activities in order to provide scientific evidence for its traditional use in the treatment of hepatitis.

Marsilea minuta Linn (Marsileaceae) is usually found at the edges of ponds and irrigation channels and as a weed in wet rice fields and it is found throughout India [4]. *Marsilea minuta* has traditional medicinal value. Traditionally the plant is used to stop nose bleeding, treat indigestion, used in kidney infection; as diuretic, anti-toxic, in hepatitis [5], in diabetes [6] etc. *Marsilea minuta* was reported for anti-fertility activity [7], antibacterial activity *invitro* [8], anxiolytic activity [9], sedative and anticonvulsant activity [10], anti-inflammatory and analgesic activity [11], antidepressant activity [12], adaptogenic and antistress activity [13] and Hypocholesterolemic [14] activities.

The plant also reported to contain Marsiline, an ester of 1-triacontanol and hexacosanoic acid [10], Marsileagenin-A, a new hexahydroxy triterpenoid sapogenol [15], flavonoids viz quercetin-3-O-glucoside, quercetin-3-O-galactoside, kaempferol-3-O-glucoside, chalcone-O-glucoside [16], Quercetin-3-rutinoside (rutin) and naringinin-7-O-glucoside [14]. The plant also contains

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ß-sitosterol and hentriacontane [4]. It is well documented that flavonoids have antioxidant property [17] which plays a vital role in the treatment of liver damage. Anti-inflammatory property of the plant may be useful inducing liver protection activity [18] and the plant has not been screened pharmacologically for its traditional claim for the treatment of hepatitis. In view of above claims and facts, the present investigation was undertaken with a view to provide scientific evidence for its traditional use in the treatment of liver disorders. The methanolic extract of plant *Marsilea minuta* was evaluated for hepatoprotective and antihepatotoxic activities against carbon tetrachloride induced hepatic damage.

MATERIALS AND METHODS

Plant Material: Fresh plants of *Marsilea minuta* Linn was collected in the month of July from rice fields of Warangal andhra Pradesh, India, after its authentication by Prof.V.S.Raju at the department of Botany, K.U, Warangal. A voucher specimen (KU/UCPSc/20/2010) of the plant is being maintained in the herbarium of University College of Pharmaceutical Sciences, K.U, Warangal.

Preparation of Extract: The whole plant was collected, dried and coarsely powdered (220grams). The powder was extracted with methanol by maceration until exhaustion. The extract was dried under reduced pressure and stored in a desiccator and stored properly until used. The yield of extract was 13% w/w.

Animals: Male Wister rats were selected for the study and maintained at a controlled temperature of 26-28°C with a 12 hr light/dark cycle and fed with a standard diet and water ad libitum. The experimental protocols were approved by the Institutional Animal Ethical Committee.

Acute Toxicity Study: Acute toxicity study was carried out for methanolic extract of *Marsilea minuta* (MMME) according to the method described in the literature [19]. Wister albino rats were divided into groups comprising six animals in each group. Rats were orally treated with graded doses (100, 500, 1000 and 2000 mg/kg) of extract ranging up to 2000 mg/kg body weight. They were observed for signs of toxicity and mortality for 72 hrs.

Chemicals: Silibinin was obtained from Sigma Aldrich, China. The biochemical analytical kits Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total Bilirubin (TB),

Direct Bilirubin (DB), Albumin (ALB) and Total Protein (TP) were purchased from Merck Specialties Private Limited, Mumbai, India. All other chemicals and solvents used were of analytical grade.

Assessment of Hepatoprotective Activity: The assessment of hepatoprotective activity was done according to the procedure given in the literature with minor modifications [20]. Male Wister rats were divided into six groups of six animals each. Group I was kept as control group treated with vehicle, (1 ml/kg b.w.p.o of 2% gum acacia in water) daily for seven days. The animals of group II also received vehicle, daily for seven days. Animals of groups III received Silibinin (50mg/kg.b.w.p.o) for seven days. Animals of groups IV, V and VI received MMME at a dose of 100, 200 and 400mg/kg b.w.p.o. respectively for seven days. On seventh day 2 hrs after the treatment, groups II, III, IV, V and VI received carbon tetrachloride (CCl$_4$) 1:1 in olive oil 1ml/kg b.w.p.o. After 24 hrs following CCl$_4$ administration, the blood was collected from the retro orbital plexus of the rats of all groups under ether anesthesia. The blood samples were allowed to stand for 30 min at room temperature and then centrifuged (Remi, model: R8-C, India) at 3000 rpm for 15 min to separate the serum. The serum was analyzed for various biochemical parameters such as AST, ALT, ALP, TB, DB, ALB and T.P. Their percentage protection was calculated by using following formula.

$$\text{Percentage protection} = \left\{ 1 - \frac{T-V}{C-V} \right\} \times 100$$

Whereas "T" is the mean value of test group (extract /standard), "C" is the mean value of toxic group (CCl$_4$) alone and "V" is the mean value of control group (vehicle treated animals). The livers were carefully removed and a part of the liver sample was preserved in 10%formalin solution for histopathological studies.

Determination of Serum Biochemical Parameters: The biochemical parameters such as AST, ALT, ALP, TB, DB, ALB and TP levels were estimated by using biochemical analytical kits using autoanalyser (Selectra Junior, vital scientific, Netherlands).

Assessment of Anti-Hepatotoxic Activity: The assessment of antihepatotoxic activity was done according to the procedure given in the literature with minor modifications [21]. Male Wistar rats were divided into six groups of six animals each. Group I was kept as control group treated with vehicle, (1 ml/kg b.w. of 2% gum acacia in water)

daily for seven days starting from 4th day to 10th day of the study. The animals of Group II served as toxic and administered orally a single daily dose of 1:1 CCl_4 in olive oil 1ml/kg b.w.p.o. on 1st and 3rd day and a single dose of 2% gum acacia from 4th day to 10th day. The animals of group III to VI were treated with CCl_4 on 1st and 3rd day and from 4th day to 10th day, group III, IV, V and VI were given orally a single daily dose of Silibinin (50 mg/kg b.w), MMME 100 mg/kg b.w., 200 mg/kg.b.w. and 400 mg/kg b.w. suspended in 2% gum acacia respectively. After 24 h of last treatment, blood and liver samples were collected from the animals of all groups for estimation of various biochemical parameters (AST, ALT, ALP, TB, DB, ALB and T.P) and histopathological studies respectively in a similar way as described under hepatoprotective activity.

Determination of Effect of Methanolic Extract (MMME) on Barbiturates Sleeping Time in Ccl₄ Induced Hepatotoxicity in Rats: The effect of MMME on pentobarbital induced sleeping time in CCl_4 intoxication in rats was determined according to the procedure described in literature [22]. Rats were divided into four groups of six each. Group I was kept as control and was given 2% gum acacia on first day. Group II served as toxic control and was administered with CCl_4 (1ml/kg b.w.p.o.). A single dose of Silibinin (50mg/kg b.w.p.o.) was administered to group III while MMME (200mg/kg b.w.p.o.) was given to group IV. After 24 h, a dose of CCl_4 (1ml/kg b.w.p.o.) was given to II, III and IV groups. Then after 2 hrs pentobarbital (25mg/kg b.w.i.p.) was given to all four groups and sleeping time was noted.

Determination of Antioxidant Activity *In vitro*
DPPH Method: Free radical scavenging activity of test extract was measured by DPPH assayed *in vitro*. [23] 0.1 mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 2.5ml of test extract suspension in water at different concentrations (10, 20, 40, 60, 80,100μg/ml). The reaction mixture was then allowed to stand at room temperature in a dark chamber for 30 min. After 30 min, absorbance was measured at 517nm on a spectrophotometer. Methanol was used as a blank and Ascorbic acid was used as a standard. The scavenging activity of DPPH radical (%) was calculated from the following equation: $[A_{Control} - A_{Extract})/A_{Control}]$ X 100.

Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The concentrations of test extract MMME were performed in triplicate and the average result was noted. The percentage inhibition was calculated by comparing the absorbance values of control and samples. [24, 25].

Statistical Analysis: The results are expressed as Mean±SD. Statistical analysis were performed by one-way analysis of variance (ANOVA) followed by Dunnett test using Graph pad Instat version 3.05,U.S.A. *P*<0.05 was considered to be significant.

Histopathology: The liver tissue was excised from the animals, washed with the normal saline to remove blood, fixed in 10% formalin for 12 hrs and processed for paraffin embedding. Thin sections were cut using rotary microtome and stained with hematoxylin and eosin for histomorphology evaluation [26].

RESULTS

The extract (MMME) did not cause any gross behavioral changes and mortality up to a dose level of 2000 mg/kg b.w. and was considered as safe. Hence three graded doses of the extract i.e., 100, 200 and 400 mg/kg b.w. were chosen for the studies. In hepatoprotective study it was observed that administration of CCl_4 elevated the levels of serum AST, ALT, ALP, TB, DB and decreased the levels of ALB and TP (Table 1),

Table 1: Effect of MMME on hepatospecific serum biochemical parameters in CCl_4 induced hepatotoxicity in rats

Groups	Dose (mg/kgp.o)	AST(U/L)	ALT(U/L)	ALP(U/L)	TBL (mg/dl)	DBL(mg/dl)	TP(g/l)	ALB(g/l)
Control		220.5±5.1	45.7±1.9	227.36±6.2	0.4±0.06	0.17±.03	8.83±0.24	4.78±0.42
Toxic (CCl₄)		538.53±4.8**	133.78±4.3**	654.45±7.1**	2.03±0.05**	0.6±.06**	4.26±0.28**	2.06±0.14**
STANDARD (Silibinin)	50	318.35±6.7** (70.3%)	59.45±7.7** (84.4%)	349.7±6.2** (72.4%)	0.55±0.07** (90.8%)	0.26±.02** (79.15)	8.25±0.39* (87.4%)	3.83±0.41** (70%)
MMME 100	100	358.2 ±6.3** (56.4%)	64.03±6.11** (78.3%)	429.18±6.8** (52.7%)	0.78±0.04** (76.7%)	0.31±.02** (67.5%)	7.28±0.25** (66.1%)	3.85±0.18** (66%)
MMME 200	200	330.7±8.4** (66.7%)	57.85±4.4** (86.3%)	380.95±5.9** (64.1%)	0.68±0.08** (83%)	0.29±.02** (72.1%)	8.06±0.47** (83.2%)	3.9±0.37** (67.7%)
MMME 400	400	384±7.9** (49.4%)	75.7±1.68** (66%)	469.11±5.9** (44.1%)	0.83±0.047** (73.7%)	0.34±.03** (60.5%)	6.98±0.3** (59.6%)	3.55±0.12** (54.8%)

Data expressed as mean ± SD, values in parathesis indicate percentage recovery. P value- control Vs other groups; P< 0.01

Table 2: Effect of MMME on hepatospecific serum biochemical parameters in CCl$_4$ induced hepatotoxicity in rats

Groups	Dose (mg/kg.p.o)	AST(U/L)	ALT(U/L)	ALP(U/L)	TBL (mg/dl)	DBL(mg/dl)	TP(g/l)	ALB(g/l)
Control		225.85±3.46**	46.01±2.52**	356.15±6.7**	0.42±0.06**	0.21±0.02**	8.5±0.3**	4.48±0.48**
TOXIC (CCl$_4$)		952.51±6.3**	133.78±4.3**	665.8±7.7**	2.05±0.08**	0.65±.04**	4.1±0.2**	2.15±0.17**
STANDARD (Silibinin)	50	422.93±6.2** (73.9%)	60.8±8.38** (83.2%)	432.41±4.32** (75.5%)	0.57±0.04* (90.8%)	0.28±.01** (84.1%)	7.8±0.29** (84.1%)	3.85±0.18** (73%)
MMME 100	100	523.3±7.14** (59.6%)	64.03±6.11** (79.5%)	534.8±6.9** (43.4%)	0.81±0.05** (76.1%)	0.32±.02** (75%)	7.75±0.34** (83%)	3.65±0.18** (64.4%)
MMME 200	200	449.36±5.64** (69.3%)	59.85±3.37** (85.1%)	491.1±8.5** (56.3%)	0.71±0.05** (82.3%)	0.3±.02** (80.6%)	8.01±0.26** (88.9%)	3.7±0.12** (66.6%)
MMME 400	400	523.8±7.12** (59%)	76.28±2.05** (66.5%)	569.2±5.68** (31.1%)	1.00±0.17** (64.7%)	0.37±.03** (64.7%)	6.68±0.26** (56.9%)	3.3±0.10** (49.4%)

Data expressed as mean ± SD, values in parathesis indicate percentage recovery. P value- control Vs other groups, P<0.05*; P< 0.01**
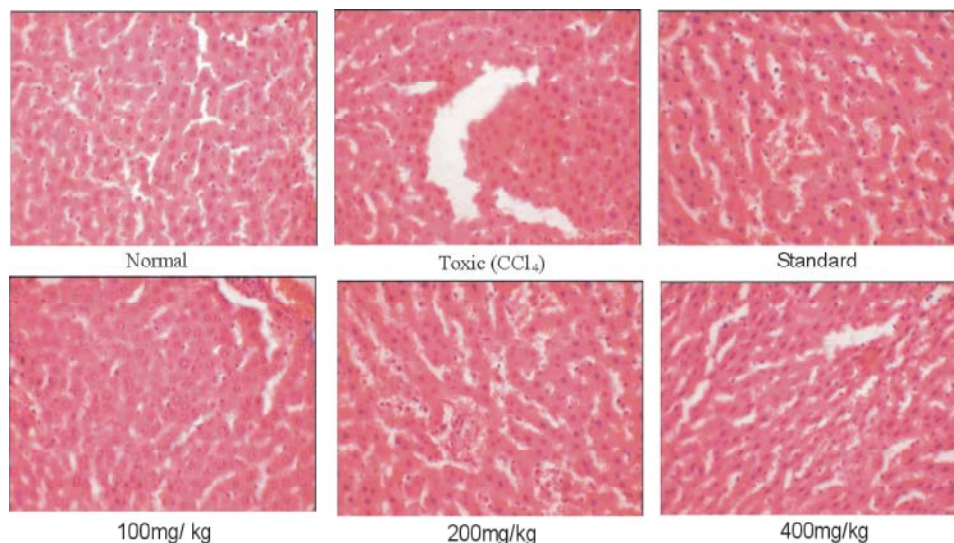

Fig. 1: Effect of MMME on histopathological changes in CCl$_4$ induced hepatotoxicity in rats. Haematoxylin and eosin stain, 45 X magnification.

indicating acute hepatocellular damage and biliary obstruction. The rats treated with standard drug Silibinin (50mg/kg), MMME 100, 200 and 400 mg/kg b.w., significantly (*P*<0.01) reversed the level of these parameters as compared to toxic group. The results indicate that MMME at 200 mg/kg. b.w. afforded better protection as compared to MMME at 100 and 400 mg/kg and is comparable to that of the reference drug Silibinin (50mg/kg). Further the results were also substantiated by the histopathological examination of liver sections (Figure 1) where CCl$_4$ intoxicated rats revealed remarkable changes in normal liver architecture of rats showing massive fatty changes, necrosis, infiltration of lymphocytes, ballooning degeneration, dilatation in sinusoidal spaces, bleeding area in hepatic lobes. The liver section of rats pretreated with MMME 100, 200, 400 mg/kg showed a significant recovery from the CCl$_4$ induced hepatic damage. Of these MMME 200 mg/kg

showed a significant recovery with little lymphocytes infiltration and dilatation of sinusoidal space. The recovery effect of MMME 200 mg/kg was comparable to that of Silibinin (50 mg/kg).

In antihepatotoxic study it was observed that administration of Ccl$_4$ significantly (*P*<0.01) elevated the levels of serum AST, ALT, ALP, TB and DB levels and decreased the levels of TP and ALB in serum, as compared to control group. The results of biochemical parameters of antihepatotoxic study are shown in Table 2. The standard, (Silibinin 50mg/kg), MMME 100, 200 and 400 mg/kg b.w., significantly (*P*<0.05) reversed the levels of these parameters in their respective groups as compared to toxic group. The results indicate that MMME at 200 mg/kg. b.w. afforded better protection as compared to MMME at 100 and 400 mg/kg. It is also evident that antihepatotoxic effect of the extract at 200mg/kg is almost close to that of the reference drug Silibinin (50 mg/kg).
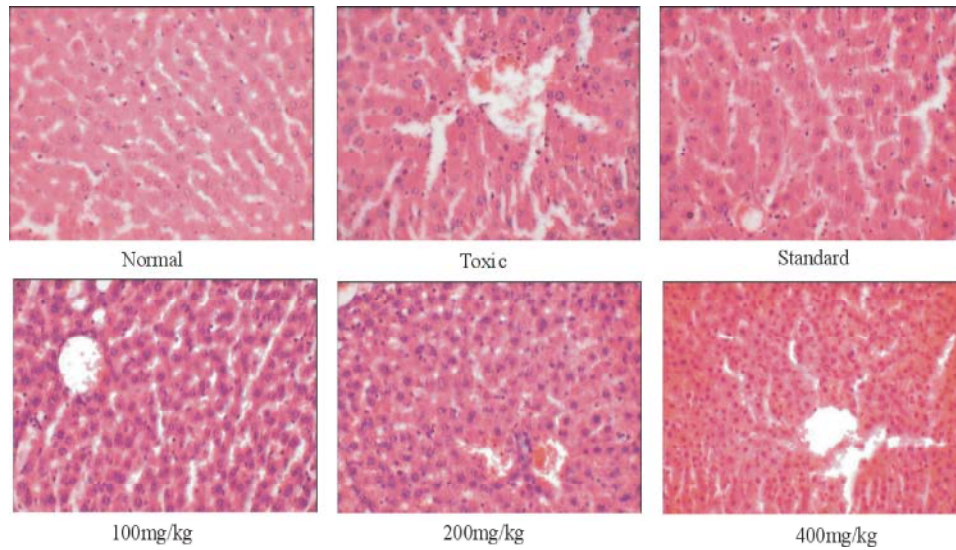
Fig. 2: Effect of MMME on histopathological changes in CCl$_4$ induced hepatotoxicity in rats. Haematoxylin and eosin stain, 45X magnification

Table 3: Effect of MMME on Pentobarbital–induced sleeping time in CCl$_4$ intoxicated rats

Group	Sleeping Time in minutes.
Control	62.35±1.74
Toxic Control	139.25±6.33**
Standard (50mg/kg)	81.90±1.48** (75%)
MMME 200	93.58±2.69** (59.4%)

Data expressed as mean ± SD, n = 6, the values in parenthesis indicate percentage recovery. *P* value CCl$_4$ vs. vehicle-<0.01; *P* value CCl$_4$ vs. treatments- <0.01**.

Fig. 3: *In vitro* DPPH radical scavenging activity of MMME.

Further the results are supported by the histopathological examination of liver sections (Figure 2), whereas CCl$_4$ intoxicated rats revealed remarkable changes in normal liver architecture of rats showing more necrosis, dilatation in sinusoidal spaces, massive fatty changes, bleeding area in hepatic lobes. Among the three test doses of the extract (MMME), rats treated with MMME 200 mg/kg showed a significant recovery from CCl$_4$ induced hepatic damage with lesser necrotic zones, ballooning degeneration and little dilatation of sinusoidal spaces which was comparable to that of reference drug Silibinin (50 mg/kg). The results of barbiturates induced sleeping time are given in Table 3. Pentobarbital at a dose of 25 mg/kg b.w.i.p. caused sedation in rats of control group for a period of 62.35±1.54 min, whereas treatment of animals with CCl$_4$ (Toxic group) significantly (P<0.01) prolonged the pentobarbital sleeping time to 139.25±6.33 min. Prior treatment of animals with MMME (200 mg/kg) and Silibinin (50 mg/kg) significantly (P<0.01)
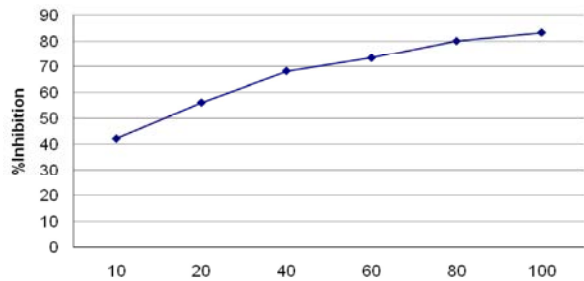
shortened the pento barbital sleeping time to 93.58±2.69 and 81.60±1.48 respectively in CCl$_4$ intoxicated rats as compared to the toxic group. The effect of MMME at 200 mg/kg. b.w. was comparable to that of reference drug, Silibinin (50 mg/kg). The 1, 1 Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity of the MMME and ascorbic acid is shown in Fig. 3. Both the Methanolic extract and ascorbic acid exhibited a concentration dependant DPPH radical scavenging activity. The IC$_{50}$ concentrations for the standard, ascorbic acid and for the methanolic extract were found to be 0.022 and 33.2 µg/ml respectively.

DISCUSSION

The plant *Marsilea minuta* (Marsileaceae) selected for the present investigation is medicinally important. It is traditionally used in the treatment of diabetes [6],

hepatitis, inflammations,[5] etc and the test tube reactions and TLC study of the extract revealed the presence of steroidal/triterpenoidal, flavonoidal compounds and their glycosides, saponins and phenolic compounds in it.

Various extracts of different parts of *Marsilea minuta* have been screened for chemicals and different pharmacological activities. It was reported that the whole plant and leaves of the plant *Marsilea minuta* not only contain chemicals of pharmacological importance such as flavonoids and sapogenin, Marsileagenin-A but also possess anti fertility activity [7], anti bacterial activity [8], anxiolytic activity [9], antidepressant activity [12], anti-stress and adaptogenic activity [13], anti-inflammatory and Analgesic activity [11] and hypocholesterolemic activity [14].

Since the plant contains antioxidant principles like flavonoids and has anti-lipidemic activity with traditional medicinal importance in hepatitis and inflammations, the present investigation was undertaken to assess scientifically the hepatoprotective potential of the plant. In this investigation, the methanolic extract of whole plant of *M.minuta* was screened for the hepatoprotective property in rats against chemical (CCl_4) induced hepatotoxicity. It was also investigated for free radical scavenging effect by DPPH method.

Ccl_4 administration in rats leads to marked elevation in the levels of serum ALT, AST and ALP. This might be due to the release of these enzymes from the cytoplasm of hepatic cells, into the blood circulation rapidly after rupture of the plasma membrane and cellular damage [27] resulting from the CCl_4 induced lipid peroxidation [28]. Treatment with MMME at 100, 200 and 400 mg/kg b.w.p.o significantly reduced the levels of these marker enzymes in CCl_4 treated rats in both hepatoprotective and antihepatotoxic studies. The decrease in the levels of these enzymes may be a consequence of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4[29].

Bilirubin is one of the most useful clinical clues to the severity of necrosis. In CCl_4 induced hepatotoxicity, elevated serum TB and DB levels are due to defective excretion of bile by the liver indicating the loss of integrity of liver and necrosis [30]. MMME at 100, 200 and 400 mg/kg b.w.p.o showed a significant decrease in serum bilirubin levels suggesting the possibility of the extract's ability to repair the damage of the hepatocytes caused by CCl_4 in prophylactic and curative studies.

In CCl_4 hepatotoxicity, a depression in TP occurs due to the disruption and dissociation of polyribosomes on endoplasmic reticulum leading to defective protein biosynthesis [31]. In both hepatoprotective and antihepatotoxic studies MMME at 100, 200, 400mg/kg increased the serum TP and ALB levels with varying degree of significance. This may be due to promotion of the assembly of ribosomes on endoplasmic reticulum to facilitate uninterrupted protein biosynthesis.

The histopathological profile of the liver of CCl_4 administered rats revealed drastic alterations in histoarchitecture showing centrilobular necrosis, fatty changes, broad infiltration of lymphocytes, ballooning degeneration and bleeding area in hepatic lobes. In hepatoprotective and antihepatotoxic studies, MMME at all test doses showed a definite sigh of protection and recovery against CCl_4 injury. Of the three test doses, MMME at 200 mg/kg exhibited a remarkable recovery towards normalization of histological architecture of liver of the rats which was almost similar to that of Silibinin (50 mg/kg).

The hepatoprotective effect of extract MMME was substantiated in pentobarbital sleeping time experiment in rats. It has been established that since the barbiturates are metabolized almost exclusively in the liver, by hepatic microsomal drug metabolizing enzymes (MDME) to inactive metabolites and any drug with an inhibitory effect on MDME is expected to prolong pentobarbital induced sleeping time. [32]. The sleeping time after a given dose is a measure of hepatic metabolism. In CCl_4 intoxication, the sleeping time after a given dose of the barbiturate will be prolonged, because the amount of hepatic metabolism/min will be less. The treatment with the extract at 200 mg/kg b.w.p.o significantly reduced the sleep time in rats which may be attributed due to the recovery of hepatic MDME. Ability of the extract MMME (200 mg/kg b.w.) to reduce the prolongation of pentobarbital-induced sleep in CCl_4 poisoned rats is further indicative of its hepatoprotective potential.

It is evident from the results that the improvement in biochemical parameters caused by MMME at 200mg/kg b.w.p.o was well supported by the improvement in histopathological findings indicating recovery of functional status of the liver by the extract. This confirms that MMME has hepatoprotective and antihepatotoxic effects in CCl_4 induced hepatotoxicity in rats by its ability to stabilize cell membrane, which may be due to its antioxidant property by invitro DPPH assay.

The antioxidant study was conducted for the methanolic extract of *Marsilea minuta* (MMME), to assess whether the antioxidant mechanism involved in its hepatoprotective activity in CCl_4 induced hepatotoxicity in rats. It has been shown that protective

agents exert their action against CCl_4 induced liver injury by impairment of Ccl_4 mediated lipid peroxidation, either through decreased production of free radical derivatives [33] or due to the antioxidant activity of the protective agent itself. In the present investigation, MMME was evaluated for antioxidant activity by DPPH method as DPPH free radical scavenging is an accepted mechanism by which antioxidants act to inhibit lipid oxidation [34]. In this study the extract MMME exhibited a concentration dependant DPPH radical scavenging activity. The maximum activity of the extract was found at the highest concentration used i.e. 100 µg/ml. Hence, the hepatoprotective activity of the extract MMME may be attributed to its antioxidant effect.

The literature reveals that the plants containing steroid and triterpenoids can control liver diseases [35]. Therefore the hepatoprotective activity of the methanolic extract of *Marsilea minuta* may be attributed due to the presence of steroid/triterpenoidal and flavonoidal glycosides and saponins in it.

REFERENCES

1. Scott tread way, 1998. An Ayurvedic Herbal Approach to a Healthy Liver. Clinical Nutrition Insights, 6(16): 1-3.

2. Radha, K.D. and K.C. Yogesh, 2005. Herbal medicines for liver diseases. Digestive Diseases and Sciences, 50(10): 1807-1812.

3. Ashis, G., 2003. Herbal folk remedies of Bankura and Medinipur District, West Bengal. Indian J. Traditional Knowledge, 2(4): 393-396.

4. Parrotta, J.A., 2001. Healing medicinal plants of peninsular India. CAB International Publishing, New York, pp: 24-25.

5. Sujan Kumar Sarker, A.B.M. and Enayet Hossain, 2009. Pteridophytes of greater mymensingh district of Bangladesh used as vegetables and medicines, Bangladesh J. Plant Taxonomy, 16(1): 47-56.

6. Sivaranjan, V.V. and I. Balachandran, 1994. Ayurvedic Drugs and their Plant Sources. Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi, pp: 455.

7. Bhardwaja, T.N. and A. Garg, 1984. The antifertility effect of an Australian species of the aquatic fern Marsilea L. Indian Fern J., 1: 75-82.

8. Parihar, P., L. Daswani and A. Bohra, 2003. Toxic effect of plant of Marsilea minuta L. on the growth of Staphylococcus aureus. Indian Fern J., 20: 48-50.

9. Bhattamisra, S.K., P.N. Singh, S.K. Singh and V. Kumar, 2007. Anxiolytic activity of Marsilea minuta Linn in rodents. Journal of Herbal Medicine and Toxicol., 1: 15-20.

10. Chatterjee, A., C.P. Dutta, B. Chaudhury, P.K. Dey, C.D. Dey, C. Chatterjee and S.R. Mukherjee, 1963. The chemistry and pharmacology of Marceline: a sedative and anticonvulsant principle isolated from Marsilea minuta Linn. and Marsilea rajastanensis gupta. J. Experimental Medical Sci. VII, pp: 53-67.

11. Bhattamisra, S.K., P.N. Singh and S.K. Singh, 2009. Anti-inflammatory and Analgesic Activity of Ethanolic extract of Marsilea minuta Linn in rodents. Drug lines in press.

12. Bhattamisra, S.K., V.K. Khanna, A.K. Agrawal, P.N. Singh and S.K. Singh, 2008. Anti depressant activity of standardised extract of Marsilea minuta Linn. J. Ethno Pharmacol., 117: 51-57.

13. Tiwari, O.P., S.K. Bhattamisra, P.N. Singh, S.K. Singh and Vikas Kumar, 2009. Adaptogenic antistress activity of Standardised extract of Marsilea minuta Linn. Pharmacology Online, 1: 290-299.

14. Gupta, R.S., P. Kumar, A. Sharma, T.N. Bhardwaja and V.P. Dixit, 2000. Hypocholesterolemic activity of Marsilea minuta Linn. in gerbils. Fitoterapia, 71: 113-117.

15. Chakravorti, D. and N.B. Debnath, 1975. Structure of Marsileagenin-A, A new hexahydroxy triterpene from M.minuta. Tetrahydron, 31: 1781-1782.

16. Wallace, J.W., M. Chapman, J.E. Sullivan and T.N. Bhardwaja, 1984. Polyphenolics of Marsileaceae and their possible phylogenic utility. American J. Botany, 71: 660-665.

17. Makari, H.K., N. Haraprasad, H.S. Patil and Ravikumar, 2008. Invitro antioxidant activity of the hexane and Methanolic Extracts of Cordia Wallichii and Celastrus Paniculata. The internet J. Aesthetic and Antiaging Medicine, 1: 1-10.

18. Vogel, G., W. Trost, R. Braatz, K.P. Odenthal, GBrusewitz, G. Antweiler and R. Seeger, 1975. Phrarmacodynamics, site and mechanism of action of silymarin, the anti hepatotoxic principle from Silibum mar, (L) Gaertn. Arzneimittelforschung, 25(1): 82-89.

19. Ghosh, M.N., 1984. In: Fundamentals of Experimental Pharmacology. Scientific Book Agency, Calcutta, II Edition, pp: 153.

20. Agarwal, M., V.K. Srivastava, K.K. Saxena and Kumar, 2006. Hepatoprotective activity of Beta vulgaris against Carbon tetrachloride induced hepatic injury in rats. Fitotherapia, 77: 91-93.

21. Rasheeduz, Z. and S. Mujahid Ali, 1998. Antihepatotoxic effects of roots and callus extracts of Cichorium intybus. J. Ethno Pharmacol., 63: 227-231.

22. Yadav, N.P. and V.K. Dixit, 2003. Hepatoprotective activity of leaves of Kalanchoe Pinnata pers. J. Ethno Pharmacol., 86: 197-202.

23. Blois, M.S., 1958. Anti-oxidant determination by the use of a stable free radical. Nature, 181: 1199-1200.

24. Harish, R. and T. Sivanandappa, 2006. Antioxidant activity and hepatoprotective potential of Phyllanthus niruri. Food Chemistry, 95: 180-185.

25. Pamitha bhandari, Neeraj Kumar, Bikram Singh, Paramvir and S.Ahuja, 2009. Online HPLC-DPPH method for antioxidant activity of Picrorhiza kurroa Royle ex Benth. and characterization of kutkoside by Ultra- Performance LC-electro spray ionization quadrupole time-of-flight mass spectrometry. Indian J. Experimental Biol., 48: 323-328.

26. Galighner, A.E. and E.N. Kozloff, 1971. Essentials of practical micro techniques. II edition, Lea and Febiger, New York.

27. Sallie, R., J.M. Tredger and R. Williams, 1991. Drugs and liver. Biopharmaceutics and Drug Disposition, 12: 251-259.

28. Azri, S., H.P. Matta, L.L. Reid, A.J. Gandlofi and K. Brendel, 1992. Further examination of selective toxicity of CCl_4 rat liver slices. Toxicology and Applied Pharmacol., 112: 81-86.

29. Kamalakkannan, N., R. Rukkumani, K. Aruna, P.S. Varma, P. Viswanthan and V. Padmanabhan, 2005. Protective effect of N-acetyl cysteine in CCl_4-induced hepatotoxicity in rats. Iranian J. Pharmacology and Therapeutics, 4: 118-123.

30. Wilson, K.J.W. and A. Waugh, 1996. Anatomy and Physiology in Health illness, VIII edition. Churchill Linngstone. New York. Edinburgh London, 306-310.

31. Clauson, G.A., 1989. Mechanism of carbon tetrachloride hepatotoxicity. Pathology and Immunopathology Res., 8: 104-112.

32. Vogel, G., 1977. Natural substances with effects on the liver. In: Wagner, H., Wolff, P. (Eds.), New Natural products and plant drugs with pharmacological, Biological or Therapeutical Activity. Springer-Verlag Berlin Heidelberg, New York, pp: 249.

33. Castro, J.A., G.C. DeFerrea, C.R. DeCastro, O.M. DeFenos, H. Sasamelt and J.R. Giltelte, 1974. Prevention of CCl_4 necrosis inhibitors of drug metabolism. Further studies on drug metabolism and their action. Biochemical Pharmacol., 23: 295-302.

34. Gulcin, I.M., E. Oktay and Kirecci E., M. Irfan Kufrevioglu O, 2002. Screening of antioxidant and antimicrobial activities of anise seed extracts. Food Chemistry, 83: 371-382.

35. Thabrew, M.I. and R.D. Hughes, 1996. Phytogenic agents in therapy in liver diseases. Phytotherapy Res., 10: 461-467.