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HEPATOPROTECTIVE POTENTIAL OF *FICUS AURICULATA* AND *SARCOCHLAMYS PULCHERRIMA*, TWO ETHNOMEDICINAL PLANTS USED BY THE MISHING COMMUNITY OF ASSAM

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ABSTRACT

Liver, the largest organ in the body is the major site of intense metabolic activities. Toxicity to the liver by various agents has been recognized as the major problem. Plant drugs practiced particularly in certain communities are playing an important role and there is a resurgence of interest in folkloric medicine for treatment of various ailments including hepatopathy and other related diseases. In the study the effect of *Ficus auriculata* and *Sarcochlamys pulcherrima*, were evaluated in carbon tetrachloride induced hepatotoxicity in rats. Liver necrosis was produced by administering carbon tetrachloride (CCl_4, 1 ml/kg, 50% v/v with olive oil, i.p.) The liver damage was evidenced by elevated levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase (ALP) and Bilirubin. The pre-treatment of both the plant extracts (200 and 400 mg/kg, p.o.) significantly ($P<0.001$) reduced CCl_4 induced elevations of the levels of SGOT, SGPT, ALP and serum Bilirubin. *Ficus auriculata* and *Sarcochlamys pulcherrima*, (400mg/kg, p.o.) and *Silymarin* (25mg/kg, p.o.), a known hepatoprotective drug showed almost similar results exhibiting hepatoprotective effect of the Plants.

KEYWORDS: Hepatoprotection, Carbon tetrachloride, Silymarin.

INTRODUCTION

Ficus auriculata Lour. Family: *Moraceae*, Mon dimoru / Altha Dimoru (Assamese.), Takuk (Mishing) and *Sarcochlamys pulcherrima* (Roxb.) Gaud. Family: *Urticaceae*, known as Mechaki (Assamese), Ombe (Mishing), are widely distributed throughout the state of Assam. The plants are also distributed all over the North East India. Flowering & fruiting generally

occur throughout the year.Young shoots, leaves and green fruits are cooked and eaten as vegetables. Ripe receptacle can be eaten, and is considered good for liver. Mishing community considers the plants as sacred plants. They make special food item from the tender leaves of the plants in almost all religious festivals, given in their traditional festival- 'Ajeng Dues'.

Oxidative damage through free radical generation is among the various mechanisms involved in the hepatotoxic effect of carbon tetrachloride (CCl_4). An anti-oxidant property is claimed to be one of the mechanisms of hepatoprotective effect of the test drug. In the present study, *Ficus auriculata* and *Sarcochlamys pulcherrima* plant extracts were investigated for its effect against CCl_4 induced hepatotoxicity in rats at a dose of 200mg/kg and 400mg/kg.

MATERIAL AND METHODS

1. Carbon tetrachloride induced hepatotoxicity in rats

The test drug of methanolic extract (ME) and aqueous extracts (AE) of both *Ficus auriculata* (FA) and *Sarcochlamys pulcherrima* (SP) were suspended in distilled water and administered at a dose of 5 ml/kg body weight orally.

2. Experimental animals

Albino Wistar rats of either sex weighing 150–200 g were used for the study.The animals were maintained on a 12 hour light and dark cycle, fed water *ad libitum.* Permission from the institutional animal ethical committee for laboratory use of animals was duly obtained from IAEC, Dibrugarh University, (IAEC/DU/112 dated. 13.2.2012).

3. Toxicity studies

Acute toxicity study was carried out using Acute Toxic Class Method as described in OECD (Organization of Economic Co-operation and Development) Guidelines No. 423.

No mortality was observed at even at maximum tested dose level i.e. 2000 mg/kg body weight of the animals. Overall results suggested the LD_{50} value of the studied plant extracts is more than 2000 mg/kg body weight to the rats.

Dose

200 mg/kg body weight and 400 mg/kg body weight of methanolic and aqueous extracts of both plants were given orally. 25 mg/kg body weight of the Standard drug Silymarin and 200 mg/kg body weight of aqueous extract of both varieties were given orally, while carbon

tetrachloride the hepatotoxic agent was given intraperitoneally at the dose of 1 ml/kg body weight mixed with 1 ml/kg,50% v/v with olive oil, i.p.

4. Hepatoprotective activity

Experimental design

The animals were classified into following groups for treatment. Group I consist of the control animals which were six in numbers. All other groups also had six animals in each of them.

Group I: Represented Vehicle control and received normal saline 5ml/kg body weight orally, daily for 9 days.

Group II: Carbon tetra chloride control group: This group received 5ml/kg of water p.o. for 9 days and carbon tetrachloride (CCl_4) 1 ml/kg, 50% v/v with olive oil, i.p. on day 7^{th} & 9^{th} day.

Group III: Animals served as standard and received silymarin 25mg/kg body weight, once daily orally for 9 days and $CCl_4$1ml/kg body weight i.p., on day 7^{th} & 9^{th}.

Group IV: Served as test and received methanolic extracts of *FA* (200 mg/kg) once daily p.o. for 9 days and CCl_4 1ml/kg, i.p., on day 7^{th} & 9^{th} day.

Group V: Served as test and received methanolic extracts of *SP* (200 mg/kg) once daily p.o. for 9 days and CCl_4 1 ml/kg, i.p., on day 7^{th} & 9^{th} day.

Group VI: Served as test and received aqueous extracts of *FA* (200 mg/kg) once daily p.o. for 9 days and CCl_4 1ml/kg, i.p., on day 7^{th} & 9^{th} day.

Group VII: Served as test and received aqueous extracts of *SP* (200 mg/kg) once daily p.o. for 9 days and CCl_4 1ml/kg, i.p., on day 7^{th} & 9^{th} day.

Group VIII: Served as test and received aqueous extracts of *FA* (400 mg/kg) once daily p.o. for 9 days and CCl_4 1ml/kg, i.p., on day 7^{th} & 9^{th} day.

Group IX: Served as test and received aqueous extracts of *SP* (400 mg/kg) once daily p.o. for 9 days and CCl_4 1ml/kg, i.p., on day 7^{th} & 9^{th} day.

On the seventh and ninth day, after two hours of the treatment in the respective groups with test and standard, 1ml/kg body weight of carbon tetrachloride in olive oil (CCl_4:olive oil,1:1) was administered intraperitoneally to each group. On the 10^{th} day i.e. 24 hour after CCl_4 administration all the animals were sacrificed with excess ether anesthesia. Blood samples were collected from rats by cardiac puncture and are allowed to clot for 30 minutes. The serum was separated by centrifugation at 5000 rpm for 20 minutes, and used for the

estimation of various serum biochemical parameters such as SGOT, SGPT, ALP and serum bilirubin by using respective test kit. The liver was immediately isolated, sliced and fixed in 15% formalin for histopathological examination. The percentage of hepatoprotection was calculated.[15]

Assessment of Biochemical Parameters

Biochemical enzymes like, SGOT, SGPT (Reitman & Frankels method), ALP and Bilirubin were assayed using standard kits from Span Diagnostics Limited, India. The reagents supplied in the kits were reconstituted, mixed with serum as directed. The SGOT and SGPT were measured at 505 nm and expressed as IU/L.

The serum alkaline phosphatase was estimated by mixing with the reagent (p-nitro phenyl phosphate, magnesium, buffers and stabilizers) with serum, estimated at 405 nm and expressed as IU/L. (Working Solution was prepared by mixing 200 µl Assay Buffer, 5 µl Mg Acetate (5 mM) and 2 µlpNPP liquid substrate (10 mM)).

For total bilirubin estimation, 0.5 ml of serum was mixed with 4.0 mL of caffeine reagent. After ten minutes, 1.0 ml sulfanilic acid was added, and after further ten minutes 3.0 ml of ascorbic acid solution is added. After ten minutes, measure the absorbance at 546 nm. In blank, serum was replaced by distilled water.

Histopathological examination of Liver

The blood was collected by retro-orbital plexus. The livers were sectioned longitudinally in two halves and were kept in 15% neutral formalin solution. All livers were processed and embedded in paraffin wax (block) and sections were taken using a microtome about 4-6 micro meters in thickness. The sections were stained with hematoxylin and eosin and were observed under a computerized Leica microscope for histological changes.

Statistical Analysis[18]

The data are expressed as mean ± SEM. Statistical analysis was made by one way ANOVA followed by Dennett's t-test; pvalue of $P < 0.01$ and $P < 0.05$ are considered as statistically significant. The minimum level of significance was $P < 0.01$. Highest significant difference test has been performed with Graph pad instat demo version software.

Table 1: Effect of methanolic extract and aqueous extracts of *Ficus auriculata* and *Sarcochlamys pulcherrima* on serum enzyme and serum biochemical parameters in rats.

Group	Dose	Serum biochemical parameters			
		SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)
I	5ml/ kg	136.29±1.88	66.94±2.41	192.69±2.89	0.53±0.09
II	1ml/kg	662.23±11.31”[a]	350.58±5.67”[a]	455.35±4.52”[a]	1.41±0.62”[a]
III	25mg/kg	178.30±4.11”[a]	113.11±6.29”[a]	207.32±4.79”[a]	0.96±0.16”[a]
IV	200mg/kg	334.06±3.86”[a]	229.40±4.02”[a]	291.76±2.11”[a]	1.26±0.11”[b]
V	200mg/kg	384.93±1.91”[a]	250.03±3.27”[b]	300.04±6.22”[a]	1.31±0.04”[b]
VI	200mg/kg	312.27±2.39”[a]	217.76±4.96”[a]	279.34±3.17”[a]	1.23±0.10”[a]
VII	200mg/kg	334.61±1.76”[a]	228.44±2.91”[a]	287.19±4.37”[a]	1.26±0.07”[b]
VIII	400mg/kg	227.52±1.84”[a]	122.79±2.73”[a]	232.33±1.16”[a]	1.14±0.15”[a]
IX	400mg/kg	253.08±3.20”[a]	139.83±1.46”[a]	246.54±3.88”[a]	1.19±0.12”[a]

Values are mean ± S.E.M, (n= 6). # compared with vehicle control (Group I), * compared with carbon tetrachloride control (Group II), [a]P<0.01 and [b]P< 0.05 are considered statistically significant. Statistical analyzed by one-way ANOVA followed by Dunnett"s t-test. The minimum level of significance was P <0.01.

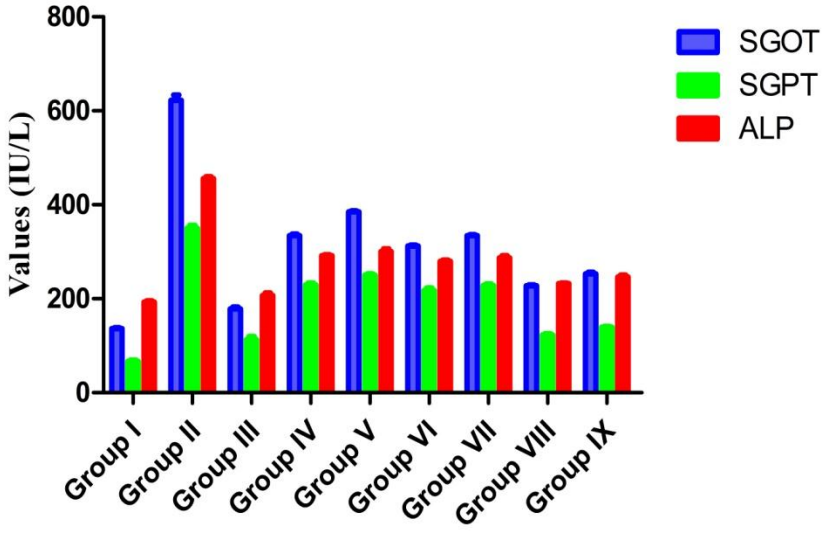

Figure: SGOT, SGPT and ALP of different groups during hepatotoxicity studies.

Histopathological (Figures) studies under Leica microscopy (H and E 100X)

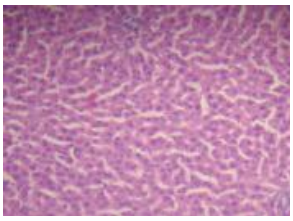

Fig. 1: Liver tissue: Normal group.

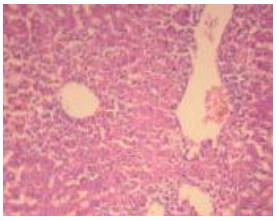

Fig 2: Liver tissue: CCl4 treated group.

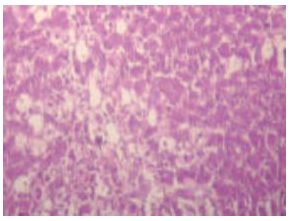

Fig 3: Liver tissue: Silymarin + CCl_4 treated group.

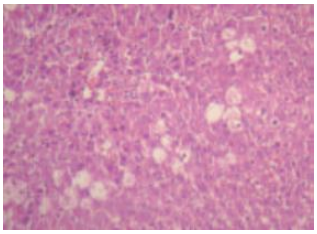

Fig. 4: Liver tissue: Test Drug (CCl_4 + ME*FA*, 200ml/kg/) treated.

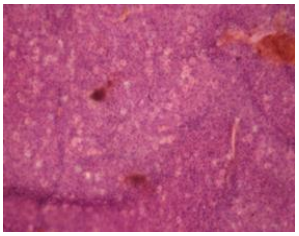

Figure 5: Liver tissue: Test Drug Treat (CCl_4 + ME*SP*, 200ml/kg/) treated.

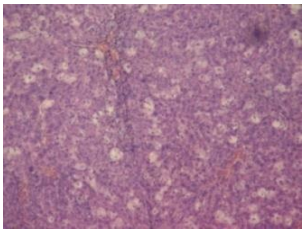

Fig. 6: Liver tissue: Test drug (CCl_4 +AE*FA* 200ml/kg).

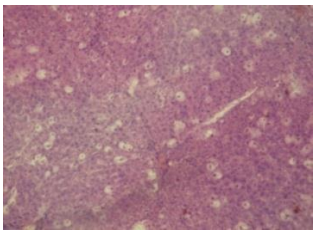

Fig. 7: Liver tissue: Test drug (CCl_4 + AE*SP* 200ml/kg).

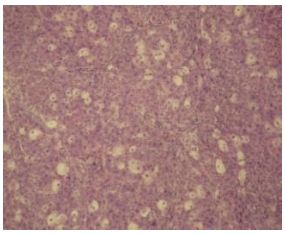

Fig. 8: Liver tissue; Test drug (CCl_4+AE*FA* 400 ml/kg).

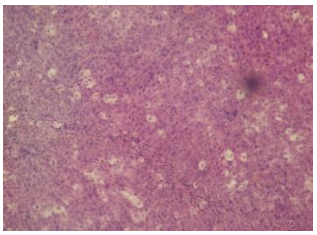

Fig. 9: Liver tissue: Test drug (CCl_4+400 AE*SP* 400ml/kg).

RESULT AND DISCUSSION

It is well known that CCL_4 is a widely used experimental hepatotoxicant. In the Living system it is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which finally binds covalently to the cell membranes and organelles to elicit lipid peroxidation, disturbing calcium ion homoeostasis and finally cell death. In the study the hepatoprotective activity is evidenced by an elevationin the serum marker enzymes namely SGOT, SGPT, ALP and Bilirubin by CCl_4 and reversal of this effect by any hepatoprotective drug. Both the plants extracts significantly reduced the elevations of liver enzymes induced by CCl_4, dose dependently. Silymarin, a prototype hepatoprotective agent also showed almost similar changes. The effects of both *Ficus auriculata* and *Sarcochlamys pulcherrima* on SGOT, SGPT, ALP and Bilirubin levels in carbon tetrachloride induced liver damage in rats are summarized in Table.1. The abnormal high level of serum biomarker enzymes and bilirubin observed in this study are the consequence of CCl_4 induced liver

dysfunction and denotes the damage to the hepatic cells. Oral administration of both plants extracts exhibited a significant reduction in CCl_4 induced levels of serum GOT, GPT, ALP and Bilirubin value remarkably to the normal group that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage.

This study exhibits the hepatoprotective effects of methanol extract and aqueous extract of both the plants in CCl_4 induced liver toxicity. Oral administration of the leaf extract of both plants at a dose of 200mg/kg and 400mg/kg body weight, markedly prevented the CCl_4 induced elevation of SGOT, SGPT, ALP and serum Bilirubin.There were significant restoration of enzyme levels on administration of methanol extract and aqueous extract at both doses (Group IV to IX) and the standard Silymarin also (Group III).

This is further evidence for the protective effect of the both the methanolic and aqueous extracts in maintaining the functional integrity of the hepatic hepatic cells. Effective control of bilirubin level and alkaline phosphatase activity points towards an early improvement in the secretary mechanism of the hepatic cell. The Silymarin with a dose of 25mg/kg, body weight has provided a better inhibition of the elevated level of SGOT, SGPT, ALP and serum bilirubin.

Administration of CCl_4(1ml/kg b.w.) and 72hrs of intoxication resulted in a significant elevation of serum marker enzymes SGPT, SGOT, ALP and Bilirubin when compared with normal control. On administration of the plants extracts with a dose of 400 mg/kg body weight has provided a better inhibition of the elevated level of SGOT, SGPT, ALP and serum bilirubin as compared with the Silymarin (Std.) group.

Treatment with both plants extracts and with CCl_4 produces lesser degree of damage to the liver cells as compared to the animals treated with CCl_4 alone.

The sections of the liver treated with (400mg/kg b.w.) of aqueous extracts and CCl_4 reveal better hepatoprotective activity than standard (Silymarin) group even that of methanolic extracts of same dose. Negligible damage to a few hepatocytes present in the close vicinity of the intralobular vein is however observed. Hepatocytes show normal appearance with the presence of vacuoles in the cytoplasm (shown in the Fig 8 and 9).

All rats except those in the control group exhibited the ballooning degeneration in the centrolobular zone and the necrosis of hepatocytes. Liver section of treated groups shows

mild fatty changes, necrosis, mild ballooning, degeneration, mild infiltration of lymphocytes and less loss of cellular boundaries with mild steatosis. The tendency of these enzymes to return towards a near normal level in the extract treated groups is a clear manifestation of anti-hepatotoxic effect of these two plants.

From the above studies, the plants clearly show the hepatoprotective activities of *Ficus auriculata* and *Sarcochlamys pulcherrima* and justify the traditional medicinal use of these plants by the Mishing community of Assam.

CONCLUSION

On the basis of various parameters undertaken, it may be concluded that *Ficus auriculata* shows more potent hepatoprotective activity than *Sarcochlamys pulcherrima.* Traditionally in different folklore medicine the aqueous juice of the *Ficus auriculata* and *Sarcochlamys pulcherrima* leaves are used for treatment of hepatic disorders. In this study we also compared the methanolic extract of the leaf and aqueous extract.

From the above result it was found that the aqueous extract of the leaves provided better result than the methanolic extract in case of both varieties, when applied at the same dose on the rats. It may be emphasized that the aqueous extract of herbal drugs at the higher dose were most effective in the study.

This study provides preliminary data on the hepatoprotective properties of the extracts from the traditionally used medicinal plants of the Mishing community of Assam and shows a good correlation with the reported traditional medical uses of these plants.

Finally it may be concluded that the *Ficus auriculata* and *Sarcochlamys pulcherrima* possesses significant hepatoprotective potential and further studies needs to be carried out for understanding the mechanism of action and its clinical applicability in hepatic disorders. However it can be assumed that the lowering of the enzyme levels particularly SGPT, SGOT, ALP and Bilirubin levels has established the hepatoprotective role of these two ethnomedicinal plants.

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