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Hepatoprotective activity of alcoholic extract of *Chonemorpha fragrans* root in against Paracetamol and Isoniazid-induced liver damage in rats

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Abstract: There is a lack of reliable hepatoprotective drugs in modern medicine to prevent and treat drug-induced liver damage. *Chonemorpha fragrans* belonging to family Apocynaceae are used traditionally for their hepatoprotective effect. We wanted to evaluate the hepatoprotective activity of *Chonemorpha fragrans* and observe whether synergistic hepato-protection exists with silymarin. The *in vitro* antioxidant and *in vivo* hepatoprotective effects of alcoholic extract of *Chonemorpha fragrans* (*C. fragrans*) root were evaluated in rats against paracetamol and Isoniazid models. The antioxidant activity of *C. fragrans* was assayed and activities were compared to standard antioxidant, ascorbic acid. The results revealed that the IC50 values of *C. fragrans* root extract for DPPH, hydroxyl, superoxide radical scavenging activities were 198.7±0.2, 275.7 ± 0.8 and 177.4±0.4 µg/mL, respectively. Liver injury was induced by paracetamol (2gm/kg) Isoniazid (100 mg/kg) orally for 14 days. The two different set of experiments the *Chonemorpha fragrans* extracts (200 and 400 mg/kg) and silymarin (25 mg/kg) were administrated orally in preventive models. The *Chonemorpha fragrans* and silymarin administration prevented the toxic effect of Paracetamol and Isoniazid the biochemical parameters in preventive model. The present study concludes that ethanolic extract of *Chonemorpha fragrans* root has significant antioxidant and hepatoprotective activity against induced Paracetamol and Isoniazid hepatotoxicity.

Keywords: Paracetamol, Isoniazid, Hepatoprotective, medicinal plants.

Introduction

Liver diseases have become one of the major causes of morbidity and mortality all over world. From among, drug induced liver injury (DILI) is one of the most common causative factor that poses a major clinical and regulatory challenge[1,2]. The manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure[4,5]. Paracetamol (PCM) and Isoniazid also known as Acetaminophen, taken in overdose can cause severe hepatotoxicity and nephrotoxicity. PCM is activated and converted by cytochrome P450 enzymes to toxic metabolite NAPQI (N-acetyl-p-benzoquinoneimine) that causes oxidative stress and glutathione (GSH) depletion. In spite of tremendous advances in modem medicine, there are hardly any reliable drugs that protect the liver from damage and/or help in regeneration of hepatic cell[2, 3]. Many active plant extracts are frequently utilized to treat a wide variety of clinical diseases including liver disease. Therefore, searching for effective and safe drugs for liver disorders are continues to be an area of interest in *Chonemorpha fragrans* (Moon) Alston (Apocynaceae)[6,7].

Part of plants used in medicine

Entire plant, roots and root bark are used for fever and stomach disorders. The plant is useful in treatment of skin diseases and inflammations[10,11]. Morphological character of *Chonemorpha fragrans* is a stout spreading laticiferous shrub with soft grayish to rusty-brown bark which yields fiber of good quality; leaves simple, opposite, large, orbicular, fulvous tomentose beneath, prominently veined; flowers large, whitish to cream-yellow, fragrant, in terminal or pseudo-auxiliary cymose panicle; fruits long, straight, woody, parallel, follicular mericarps seeds many, flat, shortly beaked with long white silky coma[12-14].

Scientific Classification of *Chonemorpha fragrans* [15]

Kingdom : Plantae,
Class : Angiospermae,
Order : Gentianales,
Family : Apocynaceae
Genus : *Chonemorpha,*
pecies : *fragrans*

Materials and methods

Chemicals

Paracetamol (PCM; Sigma-Aldrich, USA), Isoniazid and silymarin (Sigma-Aldrich) were used in the present study. All other chemicals and reagents used were of analytical grade.

Plant Material

The basic plant material of *Chonemorpha fragrans* root used for the investigation was obtain from commercial shop, Parrys, Chennai, Tamil Nadu, India. The plant can be identified authenticated by department of Botany research office (Botanist) Dr. P. Jayaraman, plant anatomy research center, Tambaram, Chennai.

Preparation of Plant Extract

The coarse powder of root of *Chonemorpha fragrans* had undergone the maceration type of extraction using methanol as the solvent system. The coarse powder of air-dried root of *Chonemorpha fragrans* was subjected to methanol extraction whereby 1 kg of powder root was macerated in 20 L of methanol in the ratio of 1 : 20 (w/v) for 72 hours, and the supernatant was filtered sequentially using cloth filter, cotton wool, and Whatman no. 1 filter paper. The solvent was then evaporated under reduced pressure (204 mbar) and controlled temperature (40°C) using a vacuum rotary evaporator (Buchi Rotavapor R210/215, Switzerland). The residue was collected and subjected to the similar extraction process for another two times.

Phytochemical Screening

The, preliminary phytochemical studies of extracts of *Chonemorpha fragrans* (Moon) Alston root confirmed the presence of desired phytochemicals were in ethanolic extracts when compared to pet.ether extract. Hence, for the pharmacological studies ethanolic extract of *Chonemorpha fragrans* (Moon) Alston root have been selected.

In vitro antioxidant activities

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity was measured according to the method of Yang et al. The reaction mixture contained 2 mL of 95% ethanol, 0.1 mol/L DPPH and 2 mL of the *Chonemorpha fragrans* extract (50–300g/mL). The mixture was incubated at 25 ◦C for 15 min, and the absorbance was determined at 517 nm. Distilled water was used as the control. The scavenging activity of DPPH radicals in samples was calculated according to the following equation-

DPPH radical scavenging activity (%)

$$= \frac{1 - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured according to the method of Winter bourn and Sutton. The reaction mixture contained 1 mL of 0.15 mol/L phosphate buffer saline (pH 7.4), 1 mL of 40 g/mL safranin, 1 mL of 0.945 mmol/L EDTA–Fe (II), 1 mL of 3% (V/V) H_2O_2, and 0.5 mL of the *Chonemorpha fragrans* extract (50–300g/mL). After incubating at 37 ◦C for 30 min, the absorbance of samples was measured at 560 nm. The IC50 value of *Chonemorpha fragrans* is the effective concentration at which the hydroxyl radicals were scavenged by 50%.

The hydroxyl radical scavenging activity was expressed as:

$$\text{Scavenging rate (\%)} \quad \frac{[A0 - A1]}{A0} \times 100$$

Where A0 was absorbance of the blank and A was the absorbance of samples

µ

Superoxide radical scavenging assay

Superoxide anion radical scavenging activity was determined according to the method of Stewart and Bewley. The reaction mixture (3 mL) contained 13mmol/L methionine, 10mmol/L riboflavin, 75 mol/L nitroblue tetrazolium, 100m mol/L EDTA, 50 m mol/L phosphate buffer (pH 7.8), and *Chonemorpha fragrans* extract (50–300g/ml). After illuminating the reaction mixture with a fluorescent lamp at 25 ◦C for 30 min, the absorbance of samples was measured at 560 nm.

The scavenging rate was calculated using the following formula:

$$\text{Scavenging rate (\%)} = \frac{[A0 - A]}{A0} \times 100$$

Pharmacological Studies

Experimental animals

The Male Sprague-Dawley rats were acclimatized for seven days under laboratory conditions before starting the experiment. Throughout the acclimatization and experimental period, the animals were housed in polypropylene cages (six rats per cage) in standard laboratory conditions (humidity 50–60%, lighting conditions (12-h light/12-h dark cycle) and temperature 21 ± 2^0C). The animals were fed with standard pellet diet and water *ad libitum*. The animals were fasted for at least 12 hours before starting the experiment.

Drugs

- Silymarin was obtained from Pfizer chemicals Pvt. Ltd., Hyderabad, India.
- A pure sample of Paracetamol was obtained from the Variety Pharmaceuticals, Kerala, India.
- A pure sample of Isoniazid was obtained from the Pfizer chemicals Pvt. Ltd., Hyderabad, India.

Test Drug

- Ethanolic extract of roots of *Chonemorpha fragrans* (Moon) Alston 200 mg/kg, 400 mg/kg.

Toxicity study

Acute toxicity relating to the determination of LD50 value was performed with different doses of the extract according to the method described by Ghosh (1984). The acute toxicity study was carried out by using Male Sprague-Dawley rats of either sex (150-200g). This study was performed as per OECD (Organization for economic co-operation and development)-423 guidelines. Animals were kept in a temp controlled environment (23 ± 2ºC) at 12 hours light/dark cycle. In the study, the drug effect was evaluated in a single dose level. The animals were divided into 3 groups (n=3) Group I (Sham Control): received 2% CMC (vehicle). Group II: received 70% ethanolic extract of C*honemorpha fragrans* root suspended in 2% CMC at a dose of 2000 mg/kg body weight orally. Group III: receives 70% ethanolic extract of C*honemorpha fragrans* root suspended in 2% CMC at a dose of 5000 mg/kg body weight orally.

Evaluation of hepatoprotective activity of ethanolic extract of roots of C*honemorpha fragrans* (moon) alston against Paracetamol induced hepatotoxicity in male sprague dawley rats.

Experimental Procedure

Male Sprague-Dawley rats were divided into five experimental groups of six rats each. Paracetamol (2 gm/kg, p.o) suspension was prepared freshly by using 1% w/v CMC and was administered to all the animals except the animals of normal control group for 14 days. Silymarin (25 mg/kg p.o) was used as standard. CF was dissolved in distilled water and administered orally once daily for a period of 14 days. Group A was maintained as normal control, which was given 1% w/v CMC. Group B received Paracetamol (2 gm/kg p.o) and served as disease control group. Group C animals were treated with Silymarin (25 mg/kg p.o) which was served as standard. Groups D and E animals were treated with two different doses of ethanolic extracts of *Chonemorpha fragrans* (Moon) Alston root 200 mg/kg and 400 mg/kg respectively. Group C, D and E were intoxicated with paracetamol (2 gm/kg) before the administration of Silymarin or Extract for 14 days. Animals were sacrificed 1 hr after treatments on 14th day. All animals were sacrificed by decapitation. The blood was collected by retro-orbital method. Blood samples were centrifuged for 10 min at 3000 rpm to separate serum. The biochemical parameters estimated according to standard methods.

Protocol

Group I - Normal control (1% w/v CMC, p.o)
Group II - Paracetamol control (Paracetamol 2gm/kg, p.o)
Group III - Paracetamol (2 gm/kg, p.o) + Silymarin (25 mg/kg, p.o)
Group IV - Paracetamol (2 gm/kg, p.o) + CF (200 mg/kg, p.o)
Group V - Paracetamol (2 gm/kg, p.o) + CF (400 mg/kg, p.o)
CF: Ethanolic Extract of Roots of *Chonemorpha fragrans* (Moon) Alston

Evaluation of hepatoprotective activity of ethanolic extract of roots of *chonemorpha fragrans* (moon) alston against isoniazid induced hepatotoxicity.

Experimental Procedure

Male Sprague-Dawley rats were divided into five experimental groups of six rats each. Isoniazid (100 mg/kg, p.o) suspension was prepared freshly by using 1% w/v CMC and was administered to all the animals except the animals of normal control group for 14 days. Silymarin (25 mg/kg p.o) was used as standard. CF was dissolved in distilled water and administered orally once daily for a period of 14 days. Group A was maintained as normal control, which was given 1% w/v CMC. Group B received Isoniazid (100 mg/kg p.o) and served as disease control group. Group C animals were treated with Silymarin (25 mg/kg p.o) which was served as standard. Groups D and E animals were treated with two different doses of ethanolic extracts of *Chonemorpha fragrans* (Moon) Alston root 200 mg/kg and 400 mg/kg respectively. Group C, D and E were intoxicated with Isoniazid (100 mg/kg) before the administration of Silymarin or Extract for 14 days. Animals were sacrificed 1 hr after treatments on 14th day. All animals were sacrificed by decapitation. The blood was collected by retro-orbital method. Blood samples were centrifuged for 10 min at 3000 rpm to separate serum. The biochemical parameters estimated according to standard methods.

Protocol

Group I - Normal control (1% w/v CMC, p.o)
Group II - Isoniazid control (Isoniazid 100 mg/kg, p.o)
Group III - Isoniazid (100 mg/kg, p.o) + Silymarin (25 mg/kg, p.o)
Group IV - Isoniazid (100 mg/kg, p.o) + CF (200 mg/kg, p.o)
Group V - Isoniazid (100 mg/kg, p.o) + CF (400 mg/kg, p.o)
EERCF: Ethanolic Extract of Roots of *Chonemorpha fragrans* (Moon) Alston

Histopathology

The livers were excised quickly and washed in ice-cold saline and fixed in 10% formalin and stained with hematoxin and eosin and then observed under microscope. The results were shown in the figure: 2,3

Statistical Analysis

All the values were expressed as mean ± SEM. (n=6 in each group). The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Tukey Krammer multiple Comparisons test. Values of $P<0.05$ was considered to be significant.

Results

The phytochemical screening of aqueous extract of *Chonemorpha fragrans*

The phytochemical tests revealed that the extract of the plant contains alkaloids carbohydrates, flavanoids, tannins and saponins (Table-1)

Effect of *Chonemorpha fragrans* extract against DPPH radicals

The free radical scavenging activity of *Chonemorpha fragrans* extract against DPPH radicals was shown in Fig. 1A. *Chonemorpha fragrans* extract and ascorbic acid standard showed antioxidant activity in a dose-dependent manner in the range of 50–500g/mL. The IC50 values for *Chonemorpha fragrans* and ascorbic acid were 198.7 and 165.2 µ g/mL, respectively.

Effects of *Chonemorpha fragrans* extract against the hydroxyl radicals

The free radical scavenging activity of *Chonemorpha fragrans* extract against hydroxyl radicals was shown in Fig. 1B. *Chonemorpha fragrans* extract and ascorbic acid standard showed antioxidant activity in a dose dependent manner in the range of 50–300 µ g/mL. The IC50 values for *Chonemorpha fragrans* extract and ascorbic acid were 275.7 and 224.2 µ g/mL, respectively.

Effect of *Chonemorpha fragrans* extract on the superoxide scavenging activity

The free radical scavenging activity of *Chonemorpha fragrans* extract against superoxide radical was shown in Fig. 1C. *Chonemorpha fragrans* extract and ascorbic acid standard showed antioxidant activity in a dose dependent manner in the range of 50–500 µ g/mL. The IC50 values for *Chonemorpha fragrans* and ascorbic acid were 177.4 and 103.6 µ g/mL, respectively.

Determination of serum biochemical parameters

Results presented (Table 2, 3) effect of ethanolic extract of roots of *Chonemorpha fragrans* (moon) alston on SGOT, SGPT, ALP, total protein, total bilirubin, direct bilirubin levels against paracetamol induced hepatotoxicity in male sprague dawley rats**.**

Table 1: Preliminary phytochemical screening of extracts of roots of *Chonemorpha fragrans* (moon) Alston

S. No	Name of the Test	Pet.Ether Extract	Ethanolic Extract
1	**Test for carbohydrates**		
	Molisch's test	-	+
	Fehling's test	-	+
	Barfoed's test	-	+
2	**Test for proteins and aminoacids**		
	Million's test	-	+
	Biuret test	-	+
	Ninhydrin test	-	+
3	**Test for alkaloids**		
	Mayer's test	-	+
	Dragendroff's test	-	+
	Wagner's test	-	+
4	**Test for glycosides**		
	Borntrager's test	-	+
	Legal's test	-	+
	Baljet's test	-	+
5	**Test for flavonoids**		
	Shinoda test	-	+
	Ferric chloride test	-	+
	Lead acetate test	-	+
6	**Test for saponins**		
	Foam test	-	+
	Haemolytic test	-	+
7	**Test for phenolic acids and tannins**		
	Ferric chloride test	-	+
	Lead acetate	-	+
	Gelatin test	-	+
8	**Test for phytosterols and triterpenoids**		
	Salkowski test	+	-
	Libermann –Burchard test	+	-
9	**Test for fixed oils and fats**		
	Spot test	-	-
	Saponification test	+	-
10	**Waxes**		
	With alcoholic KOH	+	-

Table 2: Effect of Ethanolic extract of roots of *Chonemorpha fragrans* (moon) Alston on SGOT, SGPT, ALP, total protein, total bilirubin, direct bilirubin levels against paracetamol induced hepatotoxicity in male sprague dawley rats.

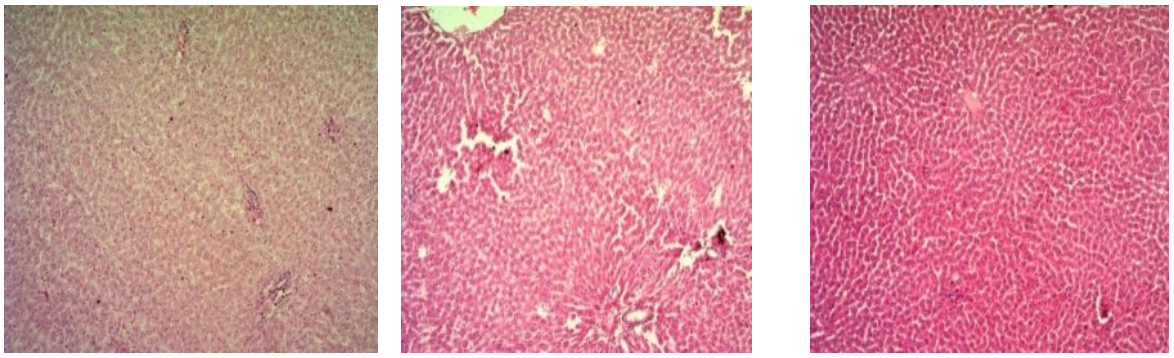
S. No	Treatment	Dose (Route)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Protein (mg/dl)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
1	Normal Control	1% w/v CMC(p.o)	51.22±0.22	27.1±0.76	62.6±0.66	6.71±0.05	0.36±0.005	0.29±0.003
2	Paracetamol Control	2 gm/kg (p. o)	178.10±0.10*	145.10±0.30*	187.61±0.64*	3.21±0.09*	1.21±0.01*	1.31±0.04*
3	Silymarin	25g/kg (p.o)	61.14±0.94*#	34.37±0.7*#	73.26±0.6*#	6.15±0.03*#	0.33±0.004*#	0.15±0.003*#
4	CF	200 g/kg (p.o)	137.23±0.84*#	91.5±0.16*#	122.17±0.75*#	4.13±0.02*#	0.811±0.006*#	0.77±0.005*#
5	CF	400 mg/kg (p.o)	69.20±0.76*#	43.5±0.12*#	78.8±0.759*#	5.23±0.05*#	0.38±0.007*#	0.27±0.002*#

n=6; values were expressed as mean ± SEM; * P<0.001 Vs normal control # P<0.001 Vs isoniazid control. Data were analyzed by One way ANOVA followed by Tukey's multiple comparison Test. Values of P<0.05 considered significant.

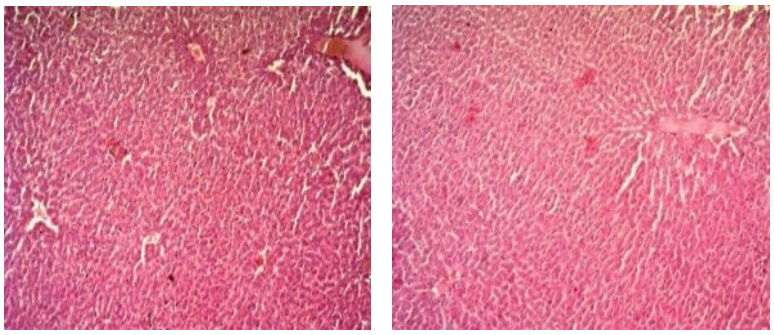
Table 3: Effect of Ethanolic extract of roots of *Chonemorpha fragrans* (moon) Alston on sgot, sgpt, alp, total protein, total bilirubin, direct bilirubin levels against isoniazid induced hepatotoxicity in male sprague dawley rats.

S. No	Treatment	Dose (Route)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Protein (mg/dl)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
1	Normal Control	1% w/v CMC (p.o)	41.33±0.88	22.5±0.76	58.5±0.76	8.71±0.07	0.36±0.005	0.29±0.003
2	Isoniazid Control	100 mg/kg (p.o)	152.67±0.66*	161.83±0.79*	176.16±0.60*	4.38±0.06*	2.49±0.06*	1.29±0.006*
3	Silymarin	25 mg/kg (p.o)	58.5±0.76*#	32.83±0.60*#	76.33±0.66*#	6.2±0.05*#	0.22±0.002*#	0.44±0.001*#
4	CF	200 mg/kg (p.o)	72.5±0.50*#	42.34±0.66*#	99.66±0.61*#	4.16±0.08*#	0.44±0.007*#	0.51±0.007*#
5	CF	400 mg/kg (p.o)	64.83±0.47*#	34.66±0.88*#	81.2±0.51*#	7.63±0.06*#	0.53±0.003*#	0.48±0.001*#

n=6; values were expressed as mean ± SEM; * P<0.001 Vs normal control # P<0.001 Vs isoniazid control. Data were analyzed by One way ANOVA followed by Tukey's multiple comparison Test. Values of P<0.05 considered significant.

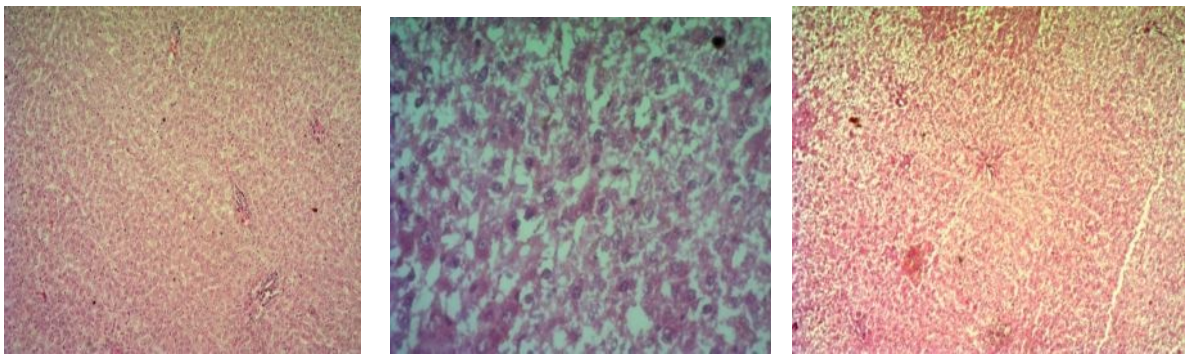


Normal Liver **Isoniazid Control (100 mg/kg)** **Silymarin (25 mg/kg p.o)**

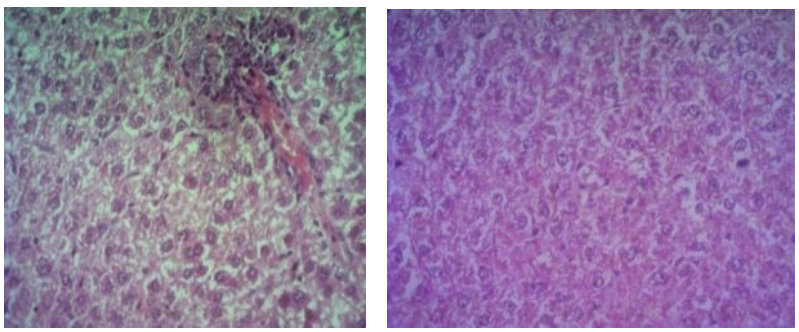


CF (200 mg/kg p.o) **CF (400 mg/kg p.o)**

Figure : 2 Effect of Ethanolic extract of roots of *Chonemorpha fragrans* (moon) alston on histopathologic sections of liver against isoniazid induced hepatotoxicity in male sprague dawley rats



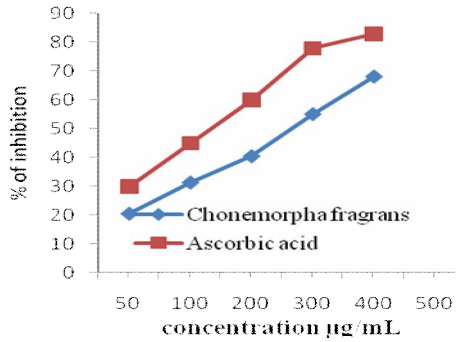
Normal Liver **Paracetamol (2gm/kg p.o)** **Silymarin(25mg/kgp.o)**



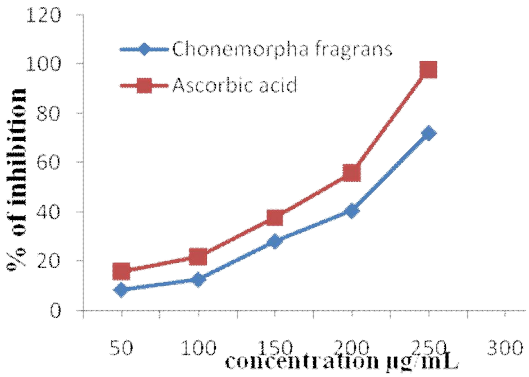
CF (200mg/kg p.o) **CF (400 mg/kg p.o)**

Figure 3: Effect of Ethanolic extract of roots of *Chonemorpha fragrans* (moon) alston on histopathologic sections of liver against paracetamol induced hepatotoxicity in male sprague dawley rats.

A. DPPH radical scavenging activity



B. Hydroxyl free radical scavenging activity



C. Super oxide radical scavenging activity

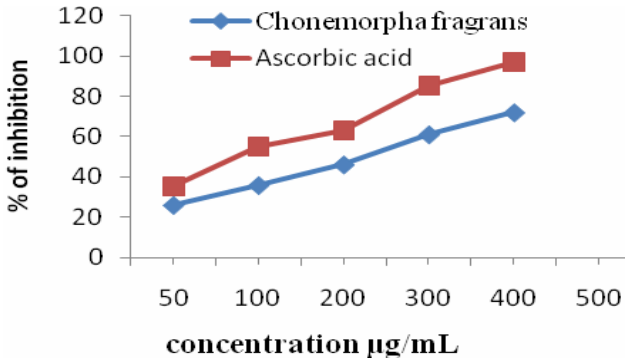


Fig. 1. DPPH, hydroxyl, super oxide radical scavenging activities of *Chonemorpha fragrans* extract and ascorbic acid

Summary

Liver diseases are among the top ten killer diseases in India. According to WHO about 18,000 people die every year due to liver diseases[16,17]. The common ailments of liver are cirrhosis, cholestasis,[21] hepatitis, portal hypertension, hepatic encephalopathy, fulminant hepatic failure and certain tumors like hepatoma[18,19]. Corticosteroids and immunosuppresants are commonly used to treat liver disease are associated with adverse effects such as immune suppression and bone marrow depression. Herbal drugs play a vital role in the management of various liver disorders; most of them speed up the natural healing process of liver[20.21].

Medicinal plants and their products are considered as less side effects and more efficacy when compared to synthetic drugs[22,25]. The alkaloids, chonemorphine present in *Chonemorpha fragrans* (Moon) Alston have shown anticancer activity in clinical research. The medicinal plant *Chonemorha fragrans* (Moon) Alston root (Apocynaceae) showed potential antioxidant and protective effects on ethanol induced oxidative damage in hepg2 cells. Based on these literature reviews, the ethanolic extract of *Chonemorpha fragrans* (Moon) Alston root was selected for screening hepatoprotective activity against paracetamol and isoniazid induced hepatotoxicity.

The extracts revealed the presence of various pharmacological active components such as; Petroleum ether extract showed the presence of steroids/triterpenoids, fixed oils, fats and waxes. Ethanol extract showed the presence of flavonoids, carbohydrates, glycosides, proteins, alkaloids. Among the two extracts the strong presence of desired phytochemicals are present in ethanolic extract. Hence, for the further studies ethanolic extract of *Chonemorpha fragrans* (Moon) Alston was selected. Although *Chonemorpha fragrans* may be described as a medicinal plant used for various purposes, no scientific reports exist on its hepatoprotective activity. The present study demonstrates for the first time the hepatoprotective activity of *Chonemorpha fragrans.*

Conclusions

The present study demonstrated that the aqueous extract of *Chonemorpha fragrans* protective against ethanol-induced hepatotoxicity which might be due to its antioxidant potential against DPPH, hydroxyl and superoxide radicals. The hepatoprotective role of *Chonemorpha fragrans* extract (400 mg/kg) was found to be comparable with Silymarin which might be due to the presence of flavonoid.

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