

An Investigation of Hepatoprotective Activity of Methanolic Extract of *Ipomoea reniformis* on Experimentally Induced Ethanol Hepatotoxicity in Rats

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Abstract

Objective: The present study evaluated the hepatoprotective activity of Methanolic Extract of *Ipomoea reniformis* (MEIR) in experimentally-induced hepatotoxic rats.

Methods: Hepatoprotective activity of MEIR was studied against ethanol (4 g/kg p.o.)-induced hepatotoxicity rats. Silymarin (100 mg/kg p.o.) was used as a standard reference. The following parameters were evaluated; serum biomarkers such as Serum Glutamate Oxaloacetic Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), total and direct bilirubin, Total Protein (TP) and tissue antioxidant levels such as Glutathione (GSH) and Lipid Peroxidation (LPO).

Results and conclusion: The control group did not exhibit increase in serum parameters, but ethanol toxicant group showed significant increase in serum parameters such as SGOT, SGPT, bilirubin (total and direct), and LPO, whereas GSH and TP levels were markedly reduced. Silymarin, MEIR low dose (200 mg/kg p.o.) and high dose (400 mg/kg p.o.) treated groups showed significant decrease in SGOT, SGPT, total and direct bilirubin, TB, LPO and increase in GSH and TP levels. Based on the study findings in serum marker enzyme levels and antioxidant parameters, it is concluded that MEIR possesses hepatoprotective activity.

Keywords: Hepatoprotection; *Ipomoea reniformis*; Ethanol; Total protein (TP)

Introduction

Scientific research in herbal medicine with hepatoprotective activity may be a great benefit as an alternative therapy in alcohol-induced liver diseases. Over consumption of alcohol has been increased and is now a serious problem in Indian society and worldwide. Three pathologically life-threatening liver diseases induced alone by alcohol abuse are fatty liver (steatosis), hepatitis and cirrhosis. The key organ for regulating homeostasis in human body is liver. It is involved with almost all the biochemical pathways related to growth, fighting against disease, nutrient supply energy provision and reproduction [1]. *Ipomoea reniformis* (IR) chois (Family: Convolvulaceae) is a perennial, much branched and procumbent herb (creeper) and also known as *Merremia emarginata*. It is widely distributed in India, Sri Lanka, Malaysia, Philippines, and Tropical Africa, and mainly grows in rainy and winter seasons. In India, it is commonly known as Undirkana and Mushakparni and it is found in southern part, especially in Chennai and few places in Andhra Pradesh [2]. IR is reported to have many important medicinal properties. In the indigenous system of medicine, IR has been claimed to be useful for cough, headache, neuralgia, rheumatism, diuretic, inflammation, troubles of nose, fever due to enlargement of liver and also in kidney diseases [3,4]. During epileptic seizures, powder of leaves is used as a snuff. In addition, juice acts as purgative, the root has diuretic and laxative properties, and also applied in the disease of the eyes and gums [5]. The major chemical

constituents of IR are p-coumaric, ferulic, sinapic acid esters and caffeic. Extract with petroleum ether contains fixed oils and fats, while aqueous extract contains tannins, starch and amino acids [6]. The MEIR has been scientifically reported with anti-inflammatory [7], antidiabetic [8], antioxidant and antiobesity [9], antiepileptic and antipsychotic activities [10]. The ethanolic extract has shown nephroprotective activity [11]. IR although used in the treatment of fever due to enlargement of liver in traditional medicines, however, there are no scientific reports for its hepatoprotective activity. On the basis of these facts, the present study was undertaken to evaluate the hepatoprotective effect of IR in rodent experimental model.

Materials and Methods

Collection of plant material

Plant material was collected from Sri Venkateshwara University Campus, Tirupati, Chittoor (district), and India. The plant was identified and authenticated by Dr. K Madhava Chetty, Assistant Professor, and Department of Botany of the same university. The herbarium (SSCP11PC0016) was prepared and kept in the Department of Pharmacology, Sree Siddaganga College of Pharmacy, Tumkur for future reference.

Preparation of methanolic extraction of *Ipomoea reniformis*

The whole plant was washed with distilled water and shade dried for two weeks. After drying, the dried plant material was powdered with

mechanical grinder and passed the powder through sieve no. 22 to get uniform particle size. The powder is subjected to defatting with n-hexane for 3 days by using Soxhlet apparatus. The plant powder (marc) was air dried after defatting. Again it is packed in Soxhlet apparatus for methanol extraction for 18 h until to get clear solution in syphon tube. Then the extract was concentrated under controlled temperature and pressure. Finally, weighed quantity of extract was used to prepare suitable doses.

Preparation of dose

Weighed quantity of MEIR was suspended in distilled water using 0.5% w/v sodium carboxy methyl cellulose (Hi-media Laboratories Pvt. Ltd., Mumbai). Based on previous studies, two doses (200 and 400 mg/kg/day) were selected [8]. In control animals, 0.5% w/v sodium carboxy methyl cellulose was served as a vehicle and administered orally. The experiments were conducted 1 h after the oral administration. In multiple dose study, the animals daily received the suitable oral dose of the MEIR for a period of 14 days. The parameters were assessed on the 15th day.

Experimental animals and research protocol approval

Young albino Wistar rats of either sex (180-220 g) were obtained from animal house of Sree Siddaganga College of Pharmacy, Tumkur (Karnataka) and maintained under controlled conditions of temperature (25°C ± 2°C) and humidity (45-60%). In addition, the animals were on a 12 h light: 12 h dark cycle and had free access to food and water ad libitum. All the animals were acclimatized for a week before the study and randomized into different groups, then housed in sanitized polypropylene cages containing sterile paddy husk as bedding. MEIR and standard drug (Silymarin) were administered once daily in the morning for a period of 14 days. Food, but not water, was withdrawn 3 h before the experiment. The protocol was approved by the Institutional Animal Ethics Committee (SSCPT/IAEC.CLEAR/106/2011-12) and the work was conducted according to CPCSEA guidelines, Govt. of India.

Assessment of hepatoprotective activity

Alcohol is known to have caloric value of about 7 calories of dietary carbohydrates so in moderate quantities; it is a food in itself except for vitamins, minerals, etc. Hence, in order to equalize the calories produced by alcohol in other groups, the animals of control group were also fed with some amount of glucose. There has been a controversy

about the type and percent of different food stuffs in diet to potentiate the severity of ethanol-induced fatty liver and hepatitis. The potentiating effect of dietary fat is not only dependent on fat consumed by rat, but it is also dependent on polyunsaturated fat. For development of experimentally-induced liver damage, dietary linoleic acid is essential [12]. Hence corn oil which is known to contain maximum oleic and linoleic acids was given throughout the experiment to all groups at a dosage of 10 ml/kg/day for 14 days [13].

Albino Wistar rats were grouped into five groups with six each and treated as: Group 1, (normal control) received 0.5% sodium CMC and corn oil (10 ml/kg/day) and glucose to the amount of alcohol for 14 days; Group 2, (ethanol control) received 99.9% ethanol 4 g/kg with corn oil (10 ml/kg/day) p.o. for 14 days; Groups 3 and 4, (low and high dose) received MEIR (200 and 400 mg/kg/day p.o.) and 99.9% ethanol (4 g/kg) with corn oil (10 ml/kg/day) for 14 days; Group 5, (silymarin control) received silymarin (100 mg/kg/day p.o.) and 99.9% ethanol (4 g/kg) with corn oil (10 ml/kg/day) for 14 days.

Assessment of liver functions

After 14 days of treatment with MEIR, on 15th day blood is collected by the puncture of retro-orbital plexus and then rats were sacrificed to collect liver which was immediately perfused with phosphate buffer solution. Serum was separated by centrifugation at 10,000 rpm in a laboratory centrifuge and assayed for Serum Glutamate Oxaloacetic Transaminase (SGOT) [14,15], serum glutamate pyruvate transaminase (SGPT) [16], direct and total bilirubin [14], and Total Protein (TP) [17] using standard method. The tissue homogenate was analysed for antioxidant parameters such as Lipid Peroxidation (LPO) and glutathione (GSH) [18-21].

Results

Control group rats treated with ethyl alcohol developed a significant hepatic damage as evidenced by elevated serum levels of hepato-specific enzymes like SGPT, SGOT, direct and total, TP, and LPO and significant decrease in GSH when compared to normal control. Pre-treatment with MEIR 200 mg/kg showed better protection. Silymarin (100 mg/kg) and MEIR 400 mg/kg showed highest protection against ethanol-induced toxicity to liver. Significant reduction was observed in elevated serum enzyme levels and LPO, and elevation in GSH in MEIR treated animals compared to toxic control animals. The study data are depicted in Tables 1 and 2.

Treatment	SGOT levels (U/L)	SGPT levels (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Total protein (mg/dl)
Normal	90.46 ± 5.85	56.28 ± 8.06	0.311 ± 0.030	0.29 ± 0.026	9.53 ± 0.122
Ethanol	137.0 ± 11.28 ^{***a}	115.9 ± 9.05 ^{***a}	0.82 ± 0.043 ^{***a}	0.89 ± 0.031 ^{***a}	3.91 ± 0.057 ^{***a}
MEIR (LD)+Ethanol	128.2 ± 9.90 ^{**b}	67.3 ± 12.00 ^{**b}	0.62 ± 0.057 ^{**b}	0.65 ± 0.082 ^{**b}	7.86 ± 0.259 ^{**b}
MEIR (HD)+Ethanol	70.98 ± 10.6 ^{***b}	51.86 ± 8.007 ^{***b}	0.44 ± 0.029 ^{***b}	0.38 ± 0.034 ^{***b}	7.75 ± 0.194 ^{***b}
Silymarin	82.98 ± 5.88 ^{***b}	57.76 ± 6.43 ^{***b}	0.38 ± 0.014 ^{***b}	0.30 ± 0.028 ^{***b}	9.13 ± 0.036 ^{***b}

Table 1: Effect of MEIR on SGOT, SGPT, T. Bilirubin, D. Bilirubin and T. Protein levels in Ethanol induced Hepatotoxic Rats. Values are expressed as mean+SEM (n=6), by one way ANOVA followed by Tukey test. Where, *represents significant at p<0.05, **represents highly significant at p<0.01, and ***represents very significant at p<0.001, compared to positive test. ^aEthanol induced hepatotoxic group was significantly different from normal control group. ^bTreated group were significantly different from ethanol-induced hepatotoxic group.

Treatment	Glutathione	LPO
Normal	0.028 ± 0.002	0.079 ± 0.001
Ethanol	0.006 ± 0.003 ^{***a}	0.149 ± 0.003 ^{***a}
MEIR (LD)+Ethanol	0.019 ± 0.003 ^{**b}	0.138 ± 0.009 ^{**b}
MEIR(HD)+ Ethanol	0.025 ± 0.005 ^{***b}	0.125 ± 0.004 ^{***b}
Silymarin	0.027 ± 0.001 ^{***b}	0.078 ± 0.011 ^{***b}

Table 2: Effect of MEIR on Glutathione and LPO in Ethanol induced Hepatotoxic rats. Values are expressed as mean±SEM (n=6), by one way ANOVA followed by Tukey test. Where, *represents significant at p<0.05, **represents highly significant at p<0.01, and ***represents very significant at p<0.001, compared to positive test. ^aEthanol induced hepatotoxic group was significantly different from normal control group. ^bTreated group were significantly different from ethanol induced hepatotoxic group.

Discussion

It is well known that hepatocytes participate in various metabolic activities. Hepatocytes follow three main pathways for ethanol metabolism to produce acetaldehyde; the microsomal ethanol oxidizing system which is located in the endoplasmic reticulum, the alcohol dehydrogenase pathway of cytosol, and catalase located in the peroxisomes. The feeding of ethanol in this is chronic which results in appearance of a form of cytochrome P-450 which differs from other cytochrome P-450 species [22]. Furthermore, administration of alcohol leads to slight depletion of GSH due to conjugation of GSH with acetaldehyde [12]. Alcohol produced marked liver damage as expected at given doses. The serum levels of SGOT, SGPT, total and direct bilirubin increased and TP significantly decreased and antioxidant parameters such as LPO increased and GSH decreased on administration of ethanol due to increased formation of lipoperoxides, conjugated dienes and malondialdehyde. Pre-treatment with MEIR showed a dose dependent protection against injurious effects of alcohol which markedly decreased the levels of SGOT, SGPT, total and direct bilirubin, and increase in TP resulting in the hindrance of the formation of hepatic free radicals. Pre-treatment with MEIR also showed restoration of depleted GSH levels to the normal and also brought down the elevated levels of LPO. No side effects were also seen in the rats during the experiment. These results suggest that MEIR possess hepatoprotective activity in rats. Studies are in progress to elucidate the components responsible for hepatoprotection.

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