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# An Investigation of Hepatoprotective Activity of Methanolic Extract of *Ipomoea reniformis* on Paracetamol Induced Hepatotoxicity in Rats

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**ABSTRACT**

**Objectives:** The main objective of the study was to evaluate investigation of hepatoprotective activity of methanolic extract of *Ipomoea reniformis* (*MEIR*) in experimentally induced paracetamol hepatotoxicity in rats. **Methods:** Hepatoprotective activity of *MEIR* was studied against Paracetamol (3 g/kg p.o.) induced hepatotoxicity in rats. Silymarin (100 mg/kg p.o.) was used as a standard reference in present study. Parameters evaluated in the study are serum biomarkers such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), total bilirubin (TB) and direct bilirubin (DB) and total protein (TP) and tissue antioxidant levels such as glutathione (GSH), lipid peroxidation (LPO). **Results and Conclusion:** The control group did not exhibit increase in serum parameters but Paracetamol (PCT) toxicant group showed increase in serum parameters such as SGOT, SGPT, Bilirubin (total and direct), LPO whereas glutathione and total protein levels were markedly reduced. Treated groups showed significant decrease in SGOT, SGPT, DB, TB, LPO and increase in GSH, TP levels were markedly increased in Silymarin, *MEIR* low dose (200 mg/kg p.o.) and *MEIR* high dose (400 mg/kg p.o.). Based on improvement in serum marker enzyme levels, antioxidant parameters, it is concluded that the *MEIR* possesses hepatoprotective activity.

## INTRODUCTION

Scientific research on herbal medicine with hepatoprotective activity may be of great benefit as an alternative therapy in liver diseases. The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges, like xenobiotics, drugs, viral infections and chronic alcoholism. If during all such exposures to the above mentioned challenges the natural protective mechanisms of the liver are overpowered, the result is hepatic injury. The commonly used analgesic and antipyretic drug which is over the counter is Paracetamol (PCT). Liver is most susceptible to the toxic effects of Paracetamol. The depletion of glutathione resulting in the accumulation of its toxic metabolite NAPQ1 causes hepatic dysfunction [1]. N-acetylcysteine (NAC) and liver transplantation are the only available treatment options for paracetamol toxicity [2]. *Ipomoea reniformis* chois (Convolvulaceae) is a perennial, much branched and procumbent herb (creeper). It is 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 [3]. It is reported to have many important medicinal properties. In the Indigenous system of Medicine, *Ipomoea reniformis* 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 [4,5]. During epileptic seizures, powder of leaves is used as a snuff. Apart from that, juice acts as purgative and the root has diuretic, laxative properties and applied in the disease of the eyes and gums [6].There are various chemical constituents in the plant such as 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 [7].The methanolic extract of this plant has proven to have anti-inflammatory [8], antidiabetic [9], antioxidant and antiobesity activities [10], antiepileptic and antipsychotic activities [11] while ethanolic extract of this plant has proven to have nephroprotective activity [12]. *Ipomoea reniformis* although used in the treatment of fever due to enlargement of liver in traditional medicines, however, there are no scientific reports of its hepatoprotective activity. Based upon all these facts, the present study was undertaken to evaluate effect of *Ipomoea reniformis* in rodent experimental model for hepatoprotective activity.

## MATERIALS AND METHODS

### Collection of Plant Material

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

### Methodology

### 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 passes the powder with 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 18hrs until to get clear solution in siphon 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 methanolic extract of *Ipomoea reniformis* (MEIR) was suspended in distilled water using 0.5%w/v sodium carboxymethyl cellulose which was supplied by Hi-media Laboratories Pvt. Ltd, Mumbai. Based on the earlier research, two doses (200 and 400mg/kg/day) were selected [9]. In control animals, 0.5% w/v sodium carboxymethyl 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 7 days. The parameters were assessed on the $8^{th}$ day.

### Experimental animals and research protocol approval

Young albino Wistar rats of either sex (180-220g) were obtained from animal house of Sree Siddaganga College of Pharmacy, Tumkur (Karnataka) and maintained under controlled conditions of temperature (25± 2°C) and humidity (45-60%). In addition, the animals were

kept 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 prototype drugs (Silymarin) were administered once daily in the morning for a period of 7 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) conducted according to CPCSEA guidelines, Govt. of India

**Assessment of hepatoprotective activity**

Albino Wistar rats were grouped into five groups with six animals in each and treated as

**Group 1**: (Control) received 0.5% CMC for 7 days

**Group 2**: (PCT control) received Paracetamol (3 g/kg p.o.) suspended with 0.5%CMC orally for 7 days

**Group 3 & 4**: (Low & high dose) received MEIR (200 & 400 mg/kg/day p.o.) and paracetamol (3 g/kg p.o.) on day 7 of the treatment period.

**Group 5:** (Silymarin control) received silymarin (100 mg/kg/day p.o.) and paracetamol (3 g/kg p.o.) on day 7 of the treatment period.

**Assessment of liver functions**

After 7 days of treatment with MEIR, on 8$^{th}$ day blood was collected from retro-orbital plexus puncture and rats were scarified and liver was collected immediately perfused with phosphate buffer solution. Serum was separated by centrifugation by subjecting to 10000 rpm in a centrifuge. Serum is collected and assayed for SGOT [13, 14], SGPT [15, 16]**,** direct bilirubin [13], total bilirubin [13] and total proteins [17] based on the standard method. The tissue homogenate was analyzed for antioxidant parameters such as LPO [18, 19] and GSH [20, 21] according to standard methods.

**RESULTS**

Rats treated with control group (Paracetamol) developed a significant hepatic damage as evidenced by elevated serum levels of hepatospecific enzymes like SGPT, SGOT, direct

bilirubin, total bilirubin and total protein and significant decrease in Glutathione when compared to normal control. Pre-treatment with MEIR 200mg/kg showed better protection. Silymarin (100 mg/kg) and MEIR 400 mg/kg showed good protection against PCT induced toxicity to liver. Significant reduction in elevated serum enzyme levels and LPO and elevation in Glutathione with MEIR treated animals compared to toxic control animals. The values are given in table no.1 and table no.2.

**Table 1: Effect of *MEIR* on SGOT, SGPT, T. bilirubin, D. bilirubin and T. Protein levels in Paracetamol induced Hepatotoxic Rats**

| Treatment | SGOT levels ( U/L ) | SGPT levels ( U/L ) | Total bilirubin (mg/dl) | Direct bilirubin (mg/dl) | Total protein (gm/dl) |
|---|---|---|---|---|---|
| Normal | 140.5 ±5.92 | 93.93±2.97 | 0.25±0.019 | 0.29±0.058 | 9.53±0.122 |
| Paracetamol | 273.2±9.68***a | 134.3±9.35*** a | 0.78±0.02***a | 0.78±0.007***a | 3.91±0.057*** a |
| *MEIR* (LD)+ Paracetamol | 240.2 ±7.87**b | 100.2±8.72** b | 0.64±0.049** b | 0.63±0.068** b | 7.86±0.259** b |
| *MEIR* (HD)+ Paracetamol | 174.7±9.07***b | 87.15±6.30*** b | 0.37±0.018*** b | 0.33±0.022*** b | 7.75±0.194*** b |
| Silymarin | 161.2±3.88***b | 76.23±6.20*** b | 0.35±0.039***b | 0.27±0.032*** b | 9.13±0.036*** b |

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. [a] Paracetamol induced hepatotoxic group was significantly different from Normal control group. [b] Treated group were significantly different from Paracetamol induced hepatotoxic group

**Table 2: Effect of *MEIR* on Glutathione and LPO in Paracetamol induced hepatotoxic rats**

| Group | Treatment | Glutathione | LPO |
|---|---|---|---|
| **1** | Normal | 0.27±0.0007 | 43.75±7.089 |
| **2** | Paracetamol | 0.07±0.001***a | 248.0±38.13*** a |
| **3** | *MEIR* (LD) + Paracetamol | 0.16±0.007**b | 183.0±14.25** b |
| **4** | *MEIR* (HD) + Paracetamol | 0.25±0.009***b | 75.53±14.33*** b |
| **5** | Silymarin | 0.24±0.011***b | 70.97±13.24*** b |

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. [a] Paracetamol induced hepatotoxic group was significantly different from Normal control group. [b] Treated group were significantly different from Paracetamol induced hepatotoxic group

## DISCUSSIONAND CONCLUSION

It is well known that hepatocytes participate in various enzymatic metabolic activities. PCT, a widely used analgesic and antipyretic are extremely safe at therapeutic doses. However, overdose of PCT has been the major cause for hospital admission for emergency liver transplantation. PCT is metabolized mainly by glucuronide (60%), sulfuric acid (35%) and cysteine (3%). A small amount of PCT undergoes CYP- mediated N-hydroxylation, to form a highly toxic metabolite, N-acetyl p- benzoquinoneimine (NAPQI). This metabolite reacts with glutathione and is rendered nontoxic. However when large doses are ingested NAPQI accumulates resulting in depletion of hepatic glutathione, which is responsible for toxic effects of PCT [22]. PCT treatment significantly increased the serum enzyme levels, namely SGOT, SGPT indicating chemical induced hepatocellular toxicity. Serum levels of these enzymes are very sensitive markers employed in the diagnosis of liver diseases. When the hepatocellular plasma membrane is damaged, the enzymes normally present in the cytosol are released into the blood stream. This can be quantified to assess the type and extent of liver injury [23]. Pre-treatment with MEIR restored the liver enzyme parameters showing a dose

dependent effect. The reduction of liver enzyme parameter, SGPT was significant and showed as a specific marker of liver injury due to toxic drugs, alcohol and virus. TP and bilirubin are related to hepatic cell damage [23]. Increase in TP and bilirubin is due to increased synthesis in the presence of increasing biliary pressure [23]. The decrease in the levels of TP and bilirubin may be due to the presence of flavonoids and their antioxidant effects which may protect the hepatic cell damage induced by paracetamol. Elevation of lipid peroxidation and deficiency of GSH which is an intrinsic anti-oxidant is an indication of hepatic damage. Along with that pre-treatment with MEIR also showed restoration of depleted GSH levels to the normal and also brought down the elevated levels of LPO. These results suggest that MEIR possesses hepatoprotective activity in rats. Studies are in progress to elucidate the components responsible for hepatoprotection.

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