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RESEARCH ARTICLE

# Evaluation and Screening of Hepatoprotective and Antioxidant Activity of "*Mussaenda Frondosa*" Root against Paracetamol Induced Hepatotoxicity in Wistar Rats

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## ABSTRACT

The present research work was an attempt to establish the possible hepatoprotective efficacy using aqueous extract of Mussaenda frondosa root against paracetamol induced hepatotoxicity in rats with an objective to prepare aqueous extract of Mussaenda frondosa roots, then to assess antioxidant property by using In-Vitro methods and to evaluate the hepatoprotective activity against paracetamol induced liver damage in rats by using In-Vivo methods and the following parameters such as Serum glumate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), Serum alkaline phosphate (ALP) and Serum total bilirubin (BIT) are tested from the collected blood of the rats used in the study at the baseline and the end of the dosing, So on comparison of the baseline and the end of the study parameters the results are concluded.

## KEYWORDS

Hepatoprotective, Hepatotoxicity, *Mussaenda frondosa* plant, Antioxidant, Liver damage, Paracetamol, Silymarin.

## 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]. The manifestations of drug-induced hepatotoxicity are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure. Paracetamol (PCM) also known as Acetaminophen, taken in overdose can cause severe hepatotoxicity and nephrotoxicity[2].

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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[2,3]. 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. Many active plant extracts are frequently utilized to treat a wide variety of clinical diseases including liver disease[4]. Several plants have been investigated and reported to possess antioxidant property and hepatoprotective activity like *Phyllanthus amarus, Terminalia chebula, Solanum nigrum, momordica charantia etc.* Similarly Traditionally *Mussaenda frondosa* Linn (Rubiaceae) commonly called as Nagavalli reported to possess number of medicinal

properties[5] Traditionally leaves are used in the treatment of jaundice, asthma, hyperacidity, fever, ulcers, leprosy, diuretic, wound, astringent, expectorant, anti inflammatory, cardiotonic, cough bronchitis, swells[6], antimicrobial[7] etc**.**

## MATERIALS AND METHOD

The roots of *Mussaenda frondosa* was procured from Tirupathi biological gardens in the month of November. The authentification was done by Prof. Madhava chetty, Dept of botany, Sri Venkateswara University, Tirupathi. Silymarin was obtained from Sigma chemicals, Mumbai and paracetamol was supplied by Granules pharmaceutical Ltd, Hyderabad, India as a gift sample. Animal feed was purchased from Sai Tirumula enterprises, Hyderabad. Kits for estimation of selected biochemical parameters such as SGOT, SGPT, ALP and BIT were purchased from Sri Balaji medical services, Visakhapatnam. Oral feeding needle was purchased from BIK instrument Ltd., Mumbai. All other Chemicals used were of analytical standard supplied from different suppliers as S.D.fine chemicals Mumbai, Sisco Laboratories Mumbai, Sigma chemicals USA and Loba chemie USA.

### Preparation of *Mussaenda Frondosa* Root Extract

*Mussaenda frondosa* root of freshly collected plant material was shade dried at room temperature and coarsely powdered in Weily mill. The powdered material 1kg of each plant was extracted with distilled water (4L) and filtrate was concentrated by climbing film evaporator at reduced pressure to give final yield.

### Animal Used

In this study male albino Wistar rats weighing between 170g-230g were used for the study. Study animal were provided and taken care by Teena labs Hyderabad. The rats were housed individually under standard conditions of constant temperature and lighting (12 hours light/ dark cycle). They had access to standard pellet diet and water with ***Ad libitum***.

### Methods Used for Evaluation of *In Vitro* Antioxidant Activity

The following methods are used for the determination of antioxidant activity[8]

1. Super oxide radical scavenging activity
2. Hydroxyl radical scavenging activity
3. Lipid peroxidation inhibition activity

#### *Procedure for Superoxide Radical Scavenging Activity*

Superoxide scavenging activity of the extract will be determined by McCord and Fridovitch method[9] which depends on light induced superoxide generation by riboflavin and the corresponding reduction of NBT. The assay mixture contained 0.3 ml of different dilutions and 0.2 ml ethylene diaminetetraacetic acid (6µM containing 3µg NaCN), 0.1 ml NBT (50 µM ), 0.05ml of riboflavin (2µM) and 2.35 ml phosphate buffer (58mM, pH7.8) to give a clear volume of 3 ml. The tubes are uniformly illuminated with incandescent light for 15 minutes and the optical density will be measured at 560 nm. The percentage inhibition by the extract of superoxide production will be evaluated by comparing the absorbance values of control and experimental tubes.

#### *Procedure for Hydroxyl Radical Scavenging Activity*

Hydroxyl radical scavenging activity will be measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the $Fe^{2+}$/EDTA/$H_2O_2$ System (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS)[10]. Fenton reaction mixture consisting 200µl of 10mM ferrous sulphate ($FeSO_4.H_2O$), 200µl of 10mM EDTA and 200µl of 10mM 2-deoxyribose will be mixed with 1.2ml of 0.1 M phosphate buffer (pH 7.4) and 200ml of plant extract. Thereafter 200µl of 10mM $H_2O_2$ will be added before incubation at $37^0$C for 4 h. Then, 1ml of this Fenton reaction mixture will be treated with 0.2ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 0.8 %

thiobarbituric acid and 1.5 ml of 20% acetic acid. The total volume will be then made to 5ml by adding distilled water and kept in an oil bath at $100^0$C for 1 hour. After the mixture had been cooled, 5ml of 15.1 v/v butanol –pyridine mixture was added. Following vigorous shaking, the tubes were centrifuged at 4000 rpm for 10min and the substances will be measured at 532nm. A control will be prepared using 0.1 ml of hydroxyl radicals by the extracts / compound will be determined by comparing the absorbance values of the control and the experimental tubes as calculated for superoxide radical assay.

***Procedure for Lipid Peroxidation Inhibition Activity***

Inhibition of lipid peroxidation will be determined by the method developed by Ohkawa[11] H et al., 1979. Rat liver tissue weighing 10 gm will be homogenized with polytron homogenizer in ice cold. Tris HCl buffer to produce 25% w/v homogenate. Then it will be centrifuged at 4000 rpm for 100mins. An aliquot of supernatant 0.1 ml will be mixed with 0.1 ml of plant extract of different concentration, followed by addition of 0.1 ml of potassium chloride (30 mM), 0.1 ml of ascorbic acid (0.06mM) and 0.1 ml of ammonium ferrous sulphate (0.16mM) and are incubated for one hour at $37^0$C. The reaction mixture will be treated with 0.2 ml of sodium dodecyl sulphate (8.1%), 1.5 ml of thiobarbituric acid (0.8%) and 1.5 ml of 20 % acetic acid (pH3.5). The total volume will then be made up to 4 ml by adding distilled water and kept in an oil bath at $100^0$c for 1 hour. After the mixture is cooled, 1ml of distilled water 5 ml of 15:1 v/v butanol – pyridine mixture are added. Following vigorous shaking, the tubes are centrifuged at 4000 rpm for 10min and the absorbance of the organic containing the thiobarbituric acid reactive substances (TBARS) will be measured at 532 nm. A control is prepared using 0.1 ml of respective vehicle in the place of plant extract / ascorbic acid. The percentage inhibition of lipid peroxidation by the extract will be determined by comparing the absorbance values of the control and the experimental tubes as calculated for superoxide radical assay

**Methods Used For Evaluation of Hepatoprotective Activity**

The hepatoprotective activity of *Mussaenda frondosa* root extract is evaluated for both prophylactic and curative studies. The selected rats are divided into 5 groups for the prophylactic study and 5 groups for the curative study with 6 rats in each group. Paracetamol, extract and silymarin were dissolved in 2% gum acacia suspension and given[12]. The dose of paracetamol to induce hepatic damage was selected as 2g/kg body weight for three days[13]. The doses of extract selected were 300 and 600 mg/kg body weight $1/10^{th}$ and $1/5^{th}$ of the $LD_{50}$ dose. The dose of silymarin used was 100 mg/ .bd .wt. The treatment protocol was summarized and given below.

**Prophylactic Study**

Group 1–Normal control: 2% w/v gum acacia suspension p.o, 1ml /kg once daily for 3 days

Group 2 – Paracetamol as toxicant 2g/kg, p.o once daily for 3 days

Group 3 – Extract 300 mg/kg p.o, 30 min. later PCM 2 g/kg p.o for 3days

Group 4 – Extract 600 mg/kg p.o, 30 min. later PCM 2 g/kg p.o for 3days

Group 5 – Silymarin 100 mg /kg p.o, 30 min. later PCM 2 g/kg p.o for 3days

**Curative Study**

Group 1– Normal control: 2% w/v gum acacia suspension p.o, 1ml /kg once daily for 10 days

Group 2 – Paracetamol as toxicant 2g/kg, p.o once daily, for 3 days

Group 3 – PCM 2 g/kg p.o for 3days followed by 300 mg/kg extract once daily p.o from $4^{th}$-$10^{th}$ day.

Group 4 – PCM 2 g/kg p.o for 3days followed by 600 mg/kg extract once daily p.o from $4^{th}$-$10^{th}$ day.

Group 5 – PCM 2 g/kg p.o for 3days followed by 100 mg /kg Silymarin p.o from $4^{th}$-$10^{th}$ day

**Methodology**

On $0^{th}$ day (baseline) blood sample is collected for all the groups of both prophylactic and curative study groups and then after the completion of dosing on $4^{th}$ day for prophylactic groups and on $11^{th}$ day for curative groups blood was collected from all animals by retro orbital puncture. Serum was separated by centrifugation (3000rpm for 15min) and subjected for estimation of biochemical parameters SGPT, SGOT, ALP and BIT is done.

**Statistical Analysis**

Results were expressed as mean ± SD. The difference among means was analysed by unpaired students T test.

## RESULTS

**Results of Antioxidant Activity of *Mussaenda Frondosa* Aqueous Extract:** As described earlier antioxidant activity of the extract was observed using three different methods by comparing extract with ascorbic acid, the observed percentage inhibition and $IC_{50}$ of all the three methods when observed with ascorbic acid and extract is tabulated in Table 1.

**Discussions on Antioxidant Activity of *Mussaenda Frondosa* Aqueous Extract:** Superoxide's are produced from molecular oxygen due to its oxidative enzymes of body as well as via non –enzymatic reaction such as auto oxidation by catecholamines. The aqueous extract of *Mussaenda frondosa* produced dose dependent inhibition of superoxide radicals. A single hydroxyl radical can result in formation of many molecules of lipid peroxides in the cell membrane which may severely disrupt its function and lead to cell death. The extract showed dose dependent activity and ascorbic acid at various concentration produced dose dependent inhibition of hydroxyl radical. Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like brain and liver. The extract showed concentration dependent prevention toward generation of lipid peroxides.

**Results Observed On Evaluation of Hepatoprotective Activity:** Both prophylactic and curative study was done for evaluation of hepatoprotective activity of the Extract taken from *Mussaenda frondosa* roots and the observations of the prophylactic study are tabulated in Table 2. And observation of curative study was tabulated in Table 3. And the discussions on the same are as follows.

**Discussions on Hepatoprotective Activity Results:** The hepatic injury leads to elevation of serum levels of SGOT (AST), SGPT (ALT), ALP and BIT in rats and are used as markers for assessing toxicant effect and also hepatoprotective agents. These parameters are also used clinically for assessing hepatic damage and effectiveness of therapeutic agents given. Hence paracetamol induced hepatotoxicity in rat represent an adequately established experimental model of liver cirrhosis (hepatic degeneration) in man and it is used for the screening of hepatoprotective drugs. The main aim of the any medication in the treatment of liver disorders is to prevent degeneration of hepatocytes and associated metabolic abnormalities and promote regeneration of hepatic cells. In the present study the hepatoprotective activity of aq. Extract of *Mussaenda frondosa* (MF) roots was evaluated in paracetamol induced liver toxicity by estimating the above mentioned biochemical parameters. Acute administration of paracetamol produced marked elevation of serum levels of the parameters in treated rats (Group II) compared to that of the control group (Group I). Prophylactic treatment and curative treatment with MF at doses of 300 and 600 mg/kg produced dose dependent reduction in PCM induced rise of the parameters. Silymarin at 100 mg/kg bd.wt significantly prevented such rise in prophylactic study. The effect of MF 600mg/kg was found to be in between the MF 300mg/kg and silymarin 100mg/kg dose in both prophylactic and curative. However the

 

Table 1: Observations of Percentage Inhibition and $IC_{50}$

| **Quantity in micrograms (µg)** | **PERCENTAGE INHIBITION AND $IC_{50}$ OF SUPEROXIDE RADICAL SCAVENGING ACTIVITY** | | **PERCENTAGE INHIBITION AND $IC_{50}$ OF HYDROXYL RADICAL SCAVENGING ACTIVITY** | | **PERCENTAGE INHIBITION AND $IC_{50}$ OF LIPID PEROXIDATION ACTIVITY** | |
|---|---|---|---|---|---|---|
| | **Extract/compound** | | | | | |
| | **Ascorbic Acid** | **Extract** | **Ascorbic Acid** | **Extract** | **Ascorbic Acid** | **Extract** |
| 10 | 18.56 | 6.32 | 4.29 | 2.32 | 7.42 | 6.48 |
| 50 | 33.93 | 14.65 | 17.44 | 11.43 | 29.64 | 16.26 |
| 100 | 49.73 | 31.22 | 29.66 | 21.62 | 42.94 | 31.94 |
| 200 | 73.46 | 43.65 | 52.43 | 43.24 | 62.66 | 42.65 |
| 300 | 79.45 | 64.26 | 67.99 | 56.49 | 74.26 | 61.24 |
| 400 | N/A | N/A | 78.22 | 66.99 | 84.48 | 70.62 |
| $IC_{50}$ | 127.12 | 229.73 | 232.5 | 288.75 | 181.88 | 253.56 |

Table 2: Observations of Prophylactic Study Biochemical Parameters.

| **Groups** | **Treatment** | **SGPT (IU/L)** | | **SGOT (IU/L)** | | **ALP(IU/L)** | | **BIT(mg/dl)** | |
|---|---|---|---|---|---|---|---|---|---|
| | | **0th day (baseline)** | **4th day** | **0th day (baseline)** | **4th day** | **0th day (baseline)** | **4th day** | **0th day (baseline)** | **4th day** |
| **1** | **Gum acacia 1ml/kg/day** | 40.66 ± 5.08 | 40.00 ± 3.40 | 96.33 ± 9.33 | 97.33 ± 11.18 | 253.16 ± 20.40 | 256.00 ± 7.93 | 0.61 ± 0.35 | 0.65 ± 0.24 |
| **2** | **PCM 2g/kg** | 41.66 ± 3.48 | 143.8 ± 10.68 | 89.16 ± 5.41 | 471.6 ± 21.9 | 265.50 ± 13.15 | 663.33 ± 45.51 | 0.66 ± 0.33 | 2.40 ± 0.26 |
| **3** | **PCM+MF 300mg/kg** | 45.33 ± 5.57 | 102.6 ± 5.92 | 94.83 ± 7.97 | 183.70 ± 5.92 | 265.15 ± 11.75 | 435.16 ± 10.63 | 0.80 ± 0.19 | 1.92 ± 0.28 |
| **4** | **PCM+MF 600mg/kg** | 41.50 ± 3.54 | 71.33 ± 3.51 | 85.50 ± 7.27 | 120.66 ± 4.07 | 253.83 ± 13.57 | 340.50 ± 13.09 | 0.75 ± 0.26 | 1.32 ± 0.50 |
| **5** | **Silymarin 100mg/kg** | 40.00 ± 3.40 | 54.83 ± 3.03 | 92.66 ± 6.19 | 104.83 ± 5.92 | 263.66 ± 11.67 | 299.20 ± 9.09 | 0.90 ± 0.14 | 1.1 ± 0.11 |

 

Table 3: Observations of Curative Study Biochemical Parameters

| Groups | Treatment | SGPT (IU/L) | | SGOT (IU/L) | | ALP(IU/L) | | BIT(mg/dl) | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0th day (baseline) | 4th day | 0th day (baseline) | 4th day | 0th day (baseline) | 4th day | 0th day (baseline) | 4th day |
| 1 | Gum acacia 1ml/kg/day | 40.66 ± 5.08 | 42.33 ± 3.40 | 96.33 ± 9.33 | 98.66 ± 13.12 | 253.16 ± 20.40 | 256.0 ± 17.93 | 0.61 ± 0.35 | 0.65 ± 0.24 |
| 2 | PCM 2g/kg | 41.66 ± 3.48 | 143.83± 10.68 | 89.16 ± 5.41 | 471.6 ± 21.9 | 265.50 ± 13.15 | 663.3 ± 45.51 | 0.66 ± 0.33 | 2.40 ± 0.26 |
| 3 | PCM+MF 300mg/kg | 45.33 ± 5.33 | 124.83± 5.92 | 97.83 ± 7.97 | 206.0 ± 4.93 | 252.33 ± 11.75 | 438.6 ± 10.63 | 0.71 ± 0.30 | 1.99 ± 0.28 |
| 4 | PCM+MF 600mg/kg | 47.50 ± 3.54 | 74.33 ± 6.71 | 98.43 ± 7.27 | 143.5 ± 6.05 | 255.00 ± 13.57 | 384.5 ± 13.09 | 0.86 ± 0.21 | 1.34 ± 0.50 |
| 5 | Silymarin 100mg/kg | 40.00 ± 3.40 | 57.00 ± 4.58 | 92.66 ± 6.19 | 119.7 ± 7.33 | 263.66 ± 11.67 | 281.0 ± 9.09 | 0.90 ± 0.14 | 1.08 ± 0.11 |

prophylactic therapy was found to be more effective than the curative therapy.

## CONCLUSION

The aqueous extract of root of *Mussaenda frondosa* was found to have hepatoprotective activity. The decrease in serum biochemical parameters (SGOT, SGPT, ALP and BIT) supported the hepatoprotective activity of the extract. The aqueous extract of MF 600 mg/kg possess 1/10$^{th}$ activity of silymarin 100mg/kg. The free radical scavenging activity and lipid peroxidation activity might be responsible for the hepatoprotective activity as paracetamol produces liver damage by free radical generation. The presence of the antioxidant present in Mussaenda might be responsible for hepatoprotective activity. The study supports the use of decoction of *Mussaenda frondosa* roots by tribal's for the treatment of hepatic disorders.

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