HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF MURRAYA KOENIGII LEAVES AGAINST PARACETAMOL-INDUCED HEPATIC DAMAGE IN RATS

Shyamala Chennaboina*1, Y. Nar Simsma Reddy2, Vinyas Mayasa1, Md. Ashwaq Hussain1

1Department of Pharmacology, Pulla Reddy Institute of Pharmacy, Annaram(V), Jinnaram(M), Medak-500013
2Department of Pharmacology, UCPSc, Kakatiya University, Warangal.

Email: shyamala0602@gmail.com

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Abstract

*Murraya koenigii* (MK) is a tropical or sub tropical plant belonging to family of Rutaceae used in cancer, inflammatory, liver disorders and blood disorders. Liver is the principle functional organ for the metabolism of different drugs and foods. In the present study, protective effect of *Murraya koenigii* methanolic leaf extract (MMK) was investigated against paracetamol induced hepatotoxicity and compared with silymarin, a standard drug. Five groups of rats (*n* = 6) were used and they were administered orally once daily with 1.0% CMC (1ml/kg, body weight), 100mg/kg silymarin (positive control), or MMK (200 and 400mg/kg) for 7 days, followed by the hepatotoxicity induction using paracetamol (2gm/kg, po).

On the 7th day, the blood samples and livers were collected and subjected to biochemical parameters like (ALP, ALT, AST), total protein, total bilirubin levels and histopathological studies. Hepatotoxic rats were pretreated with silymarin or methanolic extract of MK for seven days. Silymarin and MMK treated groups exhibited significant (*p*<0.05) decrease in ALT and AST enzyme and converse was observed in toxicant group. Histological examination of the liver tissues supported the hepatoprotective effect of the MMK and it is comparable to standard silymarin. To conclude, that the methanolic extract of leaves of *Murrya koenigii* plant possesses good hepatoprotective activity.

1. Introduction

Plants have been an important source for diverse range of bioactive molecules for medicinal activities[1]. Allopathic drugs have been a choice for wide varieties to diseases in spite of having adverse effects[2]. Still, there is a search for drugs for maximal action and minimal adverse effects. Plants are being a source for discovering the new molecular
entities for the lead molecules with fewer side effects [3]. One such plant that we are currently investigating is *Murraya koenigii* (MK), was screened for the potential pharmacological activities for hepatoprotective activity. This plant is commonly called as curry leaves, belonging to the family Rutaceae[4]. The plant have been used in the traditional medicine since long back[5].

It was used as pyritic, cancer, analgesic, anti microbial, inflammation, ulcers and in liver protection. Scientifically, *MK* has been proved to possess anti cancer, anti inflammatory, anti microbial, anti ulcer, analgesic, anti helminthic, hepatoprotective and antioxidant[6,7,8,9,10,11,12]. Interestingly, there is a link between the hepatoprotective activity with the anti-inflammatory and anti proliferative activities, which has been exerted by the leaves of *M koenigii*[6]. Our literature survey revealed that, no attempts have been made to this date to study the hepatoprotective activity of methanolic extract *M. koenigii* (MMK) leaves.

Thus, we take this opportunity to study the hepatoprotective activity of methanol extract of *M. koenigii* leaves (MMK) using the paracetamol (PCM) induced liver damage in rats as the animal model.

**Materials and methods:**

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

**2.0 Collection of Plant Material**

The leaves of *M koenigii* were collected from their local market in Hanamkonda, Telangana. A voucher specimen (SK 1985/11) was identified by botanist Dr. M D. Mustafa, Department of Botany, Kakatiya University in comparison with specimens available at the herbarium of the Laboratory of Natural Products, Kakatiya University, Warangal. The leaves were dried under shade for 7 days at room temperature, segregated, and pulverized by mechanical grinder to form coarse powder.

**2.1 Preparation of Plant Extract.**

Fine powder was subjected to soxhlet (extraction technique) for 3 hours using methanol as solvent. Extract thus obtained was washed with petroleum ether for defatting.

Percentage yield was calculated and found to be 8%, methanolic extract was used for qualitative phytochemical screening and further studies[13,14].
2.2 Acute oral toxicity studies:
Acute toxicity studies were carried out as per OECD 423 guidelines. Wistar albino mice were divided into five groups of three animals each. Extract was administered orally to different groups at the dose level of 5, 50, 300 and 2000 (mg/kg p.o.) body weights. All animals were observed for toxic symptoms and mortality for 72 h.
The toxic effects were assessed for behavioral changes like tremors, convulsions, and salvation, diarrhea and mortality within 14 days period[15].

2.3 Pharmacological Studies:

2.3.1 PCM-Induced Hepatotoxicity activity: The in vivo hepatoprotective activity of MMK was determined using the PCM (2gm/kg, po) induced hepatotoxicity test in rats. The animals were divided into 6 groups (n=6) and administered with test and PCM solutions orally as described below for seven days [16,17,18].

(i) Group I served as normal control
(ii) Group II served as standard silymarin (200mg/kg, po).
(iii) Group III served as positive control and received (1% CMC, po)
(iv) Group IV received MMK (200 mg/kg, po)
(v) Group V received MMK (400mg/kg, po)

2.4 Biochemical Studies.
On the final day, blood was removed from retro orbital route and estimated for various biochemical parameters like Alanine aminotransferase (ALT), alkaline phosphate (ALP), aspartate aminotransferase (AST) and total protein were assayed according to the standard methods and measured using the automatic biochemical analyser [19,20].

2.5 Histopathology.
On the final day the animals were sacrificed by cervical dislocation, the liver tissue were dissected out and fixed in the 10% formalin, dehydrated in gradual ethanol (50–100%). The liver tissues were cleared in xylene, and embedded in paraffin wax. The sections, which are 5-6 μm thick, were then prepared using rotary microtome and stained with hematoxylin and eosin dye for microscopic observation of histopathological changes in the liver. Liver sections was scored and evaluated according to the severity of the hepatic injury as described by WO2013088149A1.with modifications.
2.6 Statistical analysis:
The results were analyzed using graph pad prism 5. The results of each groups were compared by student t test followed by ANOVA, p<0.05 were considered as significant.

3 Results

3.1 Phytochemical screening and acute oral toxicity studies: The percentage yield was found to be 8%. Phytochemical analysis revealed the presence of alkaloids, glycosides, carbohydrates, steroids, proteins, flavonoids, tannins, phenolic compounds. No behavioral changes and mortality were observed up to 2000mg/kg.

3.2.1 Biochemical Study.
Paracetamol administration caused significant elevation in the ALT, AST, and ALP serum marker level in group pretreated with 1% CMC and standard silymarin as compared to the toxicated group (Table 2). However, oral administration of high dose MEBP (400mg/kg) and silymarin (200mg/kg) exhibited an ability to counteract the toxic effect of PCM by decreasing the level of these enzymes in a dose dependent manner.

3.2.2 In vivo Hepatoprotective Study:
In this study, gross necropsy and histopathological study of the PCM-intoxicated liver pretreated with the respective test solution showed a correlation with serum biochemical indices. Gross necropsy and histopathological study were performed on the liver to observe any irregularities or abnormalities on the structure.

Histopathologically, the non-PCM-intoxicated liver pretreated with 1% CMC (normal) shows normal lobular architecture and normal hepatic cells with well preserved cytoplasm and well defined sinusoids line and nucleus around the perivenular area (Figure 2(a)). The section of PCM intoxicated liver, demonstrates infiltration of lymphocytes, the presence of haemorrhage and extensive coagulative necrosis of the perivenular, and midzonal region with periportal sparing (Figure 2(b)). Coagulative-type necrosis of hepatocytes was observed PCM-induced liver toxicity is present predominantly in the perivenular zone (zone 3). These pathological changes were found to be lesser as the dose of MMK increased indicating the extract ability to reverse the PCM-induced intoxication (Figures 2(d)–2(e)). Table 1 shows the histopathological scoring of fibrosis solution the liver tissues pretreated with the respective test solution. Interestingly, the presence of marked necrosis, inflammation, and hemorrhage following treatment with PCM (shown by the negative control group) was reduced remarkably when pretreated with the extract or silymarin.
4.0 Discussion:

Distortion of the hepatocytes of liver will result in altering the body metabolism. Paracetamol (PCM) is an anti-pyritic drug, overdosing will result in the hepatic damage. PCM is metabolized in the liver by cytochrome P450 (cyP450) to N-acetyl-p benzoquinoneimine (NAPQI), key metabolite responsible for toxic effect of liver [21].

NAPQI activates other enzymes of CYP450, like glutathione. It conjugates with glutathione, a key enzyme responsible for anti oxidant activity in the liver, protect the liver cell from subsequent damage. PCM overdosing will result in accumulation of NAPQI and also conjugation with glutathione, leading to the oxidation and conversion of GSH to glutathione disulfide (GSSG). GSH levels are decreased in the blood plasma, resulting mitochondrial dysfunction, lipid per oxidation and development of hepatic necrosis. This mechanism of necrosis will result in elevation of liver marker enzymes like AST and ALT in the systemic circulation[22]. PCM induced hepatotoxicity is one of the established model to screen the hepatoprotective activity of the natural and synthetic compounds. The rats were intoxicated by ingestion of 2g/kg showed significant elevation of hepatic enzymes like AST and ALT. This elevated biochemical enzymes were also confirmed by the histopathological studies with massive necrotic cells around the centrilocular zone to parenchymal zone. Administration of the MMK significantly decreased the liver enzymes like AST and ALT in blood in dose dependent manner and also normalization of the hepatocytes. Extracts regenerated the liver was almost to the standard drug silymarin. MMK treated group showed an increased number of viable cells and also decrease the liver enzymes like AST and ALT.

Phytochemical screening of MMK demonstrated the presence of flavonoids, saponins, condensed tannins, and steroids[23]. In addition, the presence of high content of total phenolic compounds in the MMK was reported in previous studies[5]. The presence of flavonoids, in particular, has been confirmed earlier by Syed benazir firdaus [12]. Flavonoids have been reported to exhibit antioxidant, anti-inflammatory and hepatoprotective activities. Furthermore, condensed tannins have been suggested to possess free radical scavenging and antioxidant, anti-inflammatory, and hepatoprotective activities, while saponins have been reported also to exhibit hepatoprotective activity via modulation of its antioxidant and anti-inflammatory activities[6,7,8,9]. Taking all these reports into consideration, it is plausible to suggest that the hepatoprotective activity of MMK involved, partly, synergistic action of flavonoids, condensed tannins, and saponins. Furthermore, the MMK successfully reversed PCM induced hepatotoxic effect, which is supported by the extract ability.
to bring down the elevated levels of ALT, AST and ALP, suggesting that these biochemical restorations could be due to the extract's inhibitory effects on cytochrome P450 or/and promotion of the PCM glucuronidation[24].

MMK has been already been proven to posses anti-oxidant activity by scavenging free radicals. This could be the possible mechanistic procedure for hepatoprotective activity against the PCM induced liver toxicity[12]. Moreover, the ability to bring down the enzymes level could be associated with the ability of MMK to prevent per oxidative degradation of membrane lipids of endoplasmic reticulum that is rich in polyunsaturated fatty acids by thwarting binding of activated radicals to the macromolecules. This process could be achieved via the antioxidant activity of MEBP. Other than that, several possible mechanisms could partly be linked to the observed hepatoprotective activity of MMK. In addition, mechanisms of hepato-protection that could take place include activation of hepatic regeneration via an enhanced synthesis of protein and glycoprotein or accelerated detoxification and excretion, prevention of the process of lipid peroxidation, and stabilization of the hepatocellular membrane[25,26,27].

5.0 Conclusions

Thus, from the present studies we could confirm the presence of hepatoprotective activity of MMK, the bioactive constituents are yet to be identified and assessed for the further confirmatory mechanistic studies.

Table: 1 Values for graphical representation of histopathology results [21,22].

<table>
<thead>
<tr>
<th>Groups</th>
<th>Categorical description</th>
<th>Fibrosis measurement</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>3%</td>
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<tr>
<td>Standard</td>
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<tr>
<td>Toxic</td>
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<td>24.3%</td>
</tr>
<tr>
<td>MMK (200mg/kg)</td>
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<td>13.7%</td>
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<tr>
<td>MMK (400mg/kg)</td>
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<td>6.5%</td>
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Table: 2 Effect of MMK pretreatment on different biochemical parameters.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Protein (gm%)</th>
<th>Albumin (gm%)</th>
<th>Total Bilirubin (mg/dl)</th>
<th>Direct Bilirubin (mg/dl)</th>
<th>Ast(Sgot) (U/L)</th>
<th>Alt(Sgpt) (U/L)</th>
<th>Alkaline Phosphatase (U/L)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.2±0.62</td>
<td>2.2±0.20</td>
<td>0.15±0.03</td>
<td>0.50±0.04</td>
<td>86.45±5.40</td>
<td>42.63±5.79</td>
<td>183.1±0.3</td>
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<tr>
<td>Toxic</td>
<td>5.05±0.91</td>
<td>0.36±0.21</td>
<td>0.91±0.03</td>
<td>0.95±0.03</td>
<td>170.1±6.80</td>
<td>147.3±8.06</td>
<td>259.8±0.2</td>
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<td>GROUPS</td>
<td>Catagorical description</td>
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<td>TEST 1</td>
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Fig:1 Illustrating the histo pathology of the livers in different groups
References:

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**Corresponding Author:**

**Shyamala Chennaboina***,

**Email:** shyamala0602@gmail.com