Hepatoprotective activity of *Cyperus articulatus* Linn. against paracetamol induced hepatotoxicity in rats

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ABSTRACT

*Cyperus articulatus* L. (Cyperaceae), commonly known as piri piri, is a medicinal plant used traditionally for several medicinal purposes like headaches, migraine, epilepsy, etc. The present study evaluated the hepatoprotective activity of the methanol extract of *Cyperus articulatus* Linn. (MECA) against paracetamol induced liver damage in rats. Hepatotoxicity was induced in Wistar rats by oral administration of paracetamol (640 mg/kg suspended in 1% carboxy methyl cellulose), once during the 16 days treatment period. MECA was administered orally at the doses of 200 and 400 mg/kg daily for 16 days. Silymarin (25 mg/kg) was used as standard drug. Hepatoprotective activity was evaluated by the biochemical estimation of liver function parameters (SGPT, SGOT, ALP, total protein and total billirubin), antioxidant assays of liver homogenate (lipid peroxidation, reduced glutathione content, superoxide dismutase and catalase activity) and histological study of liver tissue. In MECA treated animals, the toxic effect of paracetamol was controlled significantly by restoration of the biochemical parameters, such as, SGPT, SGOT, ALP, total protein and total billirubin, as well as by the improvement of the antioxidant status towards near normal values. Histology of the liver sections of the animals treated with the extracts showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration, which further evidenced the hepatoprotective activity of MECA. The results show that the methanol extract of *Cyperus articulatus* possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

Keywords: *Cyperus articulatus*, Paracetamol, Hepatoprotective, Silymarin, Antioxidant.

INTRODUCTION

Herbs play a major role in the management of various liver disorders along with other system associated diseases. Liver is a key organ regulating homeostasis within the body by various functions. Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Hepatotoxicity is very common aliment resulting in serious debilities ranging from severe metabolic disorders to even mortality [1]. Plant derived natural products such as flavonoids, terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity [2, 3, 4].

*Cyperus articulatus* Linn.(Cyperaceae), commonly known as Piri piri, is a useful Indian medicinal plant which has been credited with therapeutic properties to treat several diseases like headaches, migraine, epilepsy, etc. [5]. *Cyperus articulatus* contains flavonoids, polyphenols, saponins, tannins, terpenes and sugars. Many of its biological actions are attributed to various sesquiterpenes called cyperones. The roots contain a sesquiterpene ketone, articulone, which is identical to cyperone. Two of these chemicals, called cyperotundone and alpha-cyperone, have been reported with antimalarial actions, as well as the ability to inhibit nitric oxide synthesis (a pro-oxidant) [6,7]. The decoction of rhizomes of *Cyperus articulatus* was shown to possess sedative properties in mice [6]. The root
extract has been used as carminative, antiemetic, sedative. The rural person takes aqueous extract in night for various enteral diseases and to be physically and mentally well being. The present study was undertaken to study the possible hepatoprotective role of methanol extract of rhizome of *Cyperus articulatus*.

Paracetamol (acetaminophen) is a widely used antipyretic and analgesic which produces acute liver damage if overdoses are consumed. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates [8,9]. However, the hepatotoxicity of paracetamol has been attributed to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450 [10] to a highly reactive metabolite *N*-acetyl-*P*-benzoquinone imine (NAPQI) [11]. NAPQI is initially detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid [12]. However, when the rate of NAPQI formation exceeds the rate of detoxification by GSH, it oxidizes tissue macromolecules such as lipid or SH group of protein and alters the homeostasis of calcium after depleting GSH. Silymarin is marketed as one of the standard hepatoprotective herbal formulation.

**EXPERIMENTAL SECTION**

**Plant materials**
The rhizome of *Cyperus articulatus* was collected from the rural region of Midnapore (East) West Bengal, India. The rhizome was authenticated by the Botanical Survey of India (BSI), Shibpur (W.B), India and the Voucher specimen (CNH/I-I/ (280) /2012/Tech.II/258) has been preserved in our research laboratory for our future reference. Air dried whole rhizomes (225 g) were powdered in a mechanical grinder and the powdered materials was extracted by methanol using Soxhlet extraction apparatus. The solvent was completely removed under reduced pressure in a rotary vacuum evaporator. The concentrated extract (yield 21.51%) was stored in vacuum desiccators for further use.

**Chemicals**
Paracetamol was purchased from, CIPLA Ltd., Baddi; H.P. Silymarin was supplied by Panacea Biotech Ltd, New Delhi. All other chemicals and other biochemicals used in the experiments were of analytical grade from different firms. The organic solvents were distilled before use.

**Animals**
Wistar albino rats of either sex weighing between 170-200 g were used for this purpose. The animals were housed in polypropylene cages and maintained at 24 ± 2 °C under 12h light dark cycle and were fed *ad libitum* with standard pellet diet and had free access to water maintenance and use of animals as per the experiment was approved by the institutional Animal Ethics Committee (1585/PO/a/11/CPCSEA).

**Acute toxicity**
The acute oral toxicity of MECA in male Swiss albino mice was studied as per OECD guideline 425 (OECD, 2008) [24]. LD50 value of MECA was determined using the method of maximum likelihood.

**Experimental designs**
Wistar albino rats were divided into five groups (n=6). Group I served as saline control (0.9% w/v sodium chloride, orally). Group II-V received paracetamol suspension (640 mg/kg suspended in 1% methyl cellulose; orally) once and group II served as paracetamol control. After administration of paracetamol suspension, group III and IV received MECA 200 and 400 (mg/kg body weight; orally) respectively daily for 16 days. Group V received standard drug silymarin (25 mg/kg body weight; orally) daily for 16 days. After 24 h of last dose and 18 h of fasting all animals were sacrificed by cervical dislocation. The blood and liver were collected for biochemical estimation, antioxidant assay and histopathological observation.

**Biochemical Estimation**
Serum glutamine oxaloacetate transaminase (SGOT), serum glutamine pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), total protein and total bilirubin content were estimated by using commercially available kits from Span Diagnostic Ltd., Surat, India.

**Antioxidant estimation**
For assessment of antioxidant activities, immediately after collection of blood the rats were sacrificed and livers were dissected out and washed in ice cold normal saline, blotted dry and weighed. Required quantity of the tissue was weighed and 25% (w/v) of each tissue homogenate was then prepared using KCl solution (1.15% w/v) and centrifuged at 3000 g for 1 hr. The supernatant was used for the determination of lipid peroxidation (LPO) [13] and...
endogenous antioxidant systems such as reduced glutathione (GSH) [14], superoxide dismutase (SOD) [15] and catalase [16].

**Histological observation**

Liver is dissected out and the liver samples were excised from the experimental animals of each group and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin and then with bovine solution. They were processed for paraffin embedding following the microtome technique. The sections were taken at 50 μ thickness processed in alcohol-xylene series and were stained with alum-haematoxylin and eosin. The sections were examined microscopically for the evaluation of histopathological changes.

**Statistical Analysis**

Values were presented as mean ± S.E.M. Data were statistically evaluated by one way analysis of variance (ANOVA) followed by post hoc Dunnett’s test using SPSS software. P-values of <0.001 were considered as statistically significant.

**RESULTS**

In acute toxicity study, MECA did not show any mortality or toxic effect up to the dose of 2 g/kg body weight, accordingly 200 and 400 mg/kg body weight were taken as low and high dose of MECA for the experiment.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>LPO (MDA, nano m/mg)</th>
<th>GSH (mg/mg wet tissue)</th>
<th>CAT (µM of H₂O₂ decomposed/min/mg wet tissue)</th>
<th>SOD (U/mg wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>20.75±0.75</td>
<td>5.21±0.29</td>
<td>1.04±0.04</td>
<td>10.65±0.96</td>
</tr>
<tr>
<td>Paracetamol (640 mg/kg)</td>
<td>99.31±5.59</td>
<td>2.06±0.08</td>
<td>0.47±0.025</td>
<td>6.42±0.62</td>
</tr>
<tr>
<td>MECA 200 mg/kg</td>
<td>56.56±4.58</td>
<td>3.56±0.23</td>
<td>0.75±0.015</td>
<td>8.0±0.56</td>
</tr>
<tr>
<td>MECA 400 mg/kg</td>
<td>54.95±1.76</td>
<td>3.82±0.3</td>
<td>0.8±0.01</td>
<td>8.1±0.69</td>
</tr>
<tr>
<td>Silymarin 25 mg/kg</td>
<td>39.40±4.03</td>
<td>5.1±0.22</td>
<td>0.96±0.01</td>
<td>10.3±0.92</td>
</tr>
</tbody>
</table>

Administration of paracetamol to the animals resulted in a marked elevation of serum transaminases (SGOT and SGPT), serum alkaline phosphatase (ALP) and total bilirubin (TB), when compared with those of normal control animals. However serum total protein level was decreased.

Histopathological examination of liver sections of normal control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Fig.1.A). Disarrangement of normal hepatocytes with centrilobular necrosis, vacuolization of cytoplasm and fatty changes were observed in paracetamol intoxicated rat
livers (Fig.1.B). The liver sections of the rats treated with MECA low and high dose (Fig.1.C and D) and silymarin (Fig.1.E) showed a sign of protection against paracetamol intoxication as evident by presence of normal hepatic cords and absence of necrosis with minimal inflammatory conditions around the central vein.

**DISCUSSION**

Liver is largest organ and it is target for toxicity because of its role in clearing and metabolizing chemicals through the process called detoxification. Drug induced liver disorders occurred frequently can be life threatening and mimic all forms of liver diseases [17]. Paracetamol being a drug capable of causing liver disorders if overdoses are consumed. The covalent binding of N-acetyl-P benzoquinone imine, an oxidation product of paracetamol, to
sulphhydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity have been reported earlier [18].

Estimating the activities of serum marker enzymes, like SGOT, SGPT and ALP can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol, are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage [19]. The enhanced levels of these serum marker enzymes observed in paracetamol treated rats in our study correspond to the extensive liver damage induced by the toxin. Restoration of the levels of these enzymes to/towards near normal values in the MECA, low and high dose and silymarin treated animals is a clear manifestation of anti-hepatotoxic effect of the extract and the drug. Increase in serum bilirubin in group II animals reflected the depth of jaundice, which was attenuated to the normal level in MECA treated groups, indicating its hepatoprotective effect further. The lowered level of total protein recorded in the serum of paracetamol intoxicated rats reveals the severity of hepatopathy, while the attainment of near normalcy in total protein content of serum of the treated groups potentiates the hepatoprotective effect.

The antioxidant activity or the inhibition of the generation of free radical is important in the protection against paracetamol induced liver lesion, as because, one of the principle causes of paracetamol induced liver injury is formation of lipid peroxides by free radical [20, 21]. The body has an effective defense mechanism, consisting of a set of endogenous antioxidant enzymes including SOD, catalase as well as non-enzymic antioxidants, such as reduced glutathione. In paracetamol intoxicated hepatotoxicity, the balance between ROS production and these antioxidant defenses may be lost, consequently oxidative stress may result which finally may lead to hepatic necrosis [22]. MECA treatment showed significant improvement in the level of these antioxidant systems over those in paracetamol control animals, and this clearly indicates the antioxidant activity of MECA. The level of lipid peroxide is a measure of membrane damage and alteration in structure and function of cellular membranes. Elevation of MDA level in liver indicates excessive free radical generation and consequently enhanced lipid peroxidation which leads to severe tissue damage [23], treatment with MECA significantly reversed these changes.

Histological examinations of the liver sections reveal that the normal liver architecture was disturbed by hepatotoxin intoxication. In the sections obtained from the rats treated with extract or silymarin and intoxicated with hepatotoxin, the normal cellular architecture was retained as compared to those of the normal control rats, thereby confirming the protective effect of the extract or drug and thus the observation substantiates other results of the experiment.

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