HEPATO PROTECTIVE ROLE OF TRIBULUS TERRESTRIS LINN. FRUITS ON PARACETAMOL INDUCED HEPATIC DAMAGE IN RATS

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ABSTRACT

The scientific evaluation of medicinal plants used in the preparation of folk remedies has provided modern medicine with effective pharmaceuticals for the treatment of diseases. The methanolic extract of Tribulus terrestris Linn (Zygophyllaceae) fruits was investigated for its hepatoprotective effect on paracetamol (2g/kg/b.wt/p.o) suspended in 1% CMC induced acute liver damage in Wistar albino rats. Hepato protection activity was measured by using biochemical parameters such as serum glutamate oxalate transaminase and serum Glutamate Pyruvate Transaminase (SGOT and SGPT), alkaline phosphatase (ALP), bilirubin, total protein and histopathology was also studied. The methanol extract of Tribulus terrestris (METT) at the dose of (500 mg/kg/p.o) produced significant hepatoprotective effect by decreasing the activity of serum enzymes, bilirubin and proteins. The effects of methanol extract of Tribulus terrestris were comparable to that of standard drug silymarin. These results suggest that methanol extract of Tribulus terrestris have potential therapeutic value in the treatment of some liver disorders.

KEY WORDS: Tribulus terrestris, Hepatoprotective effect, Paracetamol.

1. INTRODUCTION:

In recent years, many researchers have examined the effects of plants used traditionally by indigenous healers and herbalists to support liver function and treat diseases of the liver. In most cases, research has confirmed traditional experience and wisdom by discovering the mechanisms and mode of action of these plants as well as reaffirming the therapeutic effectiveness of certain plants or plant extracts in clinical studies. Several hundred plants have been examined for use in a wide variety of liver disorders. Just a handful has been fairly well researched (Scott, 1998). A number of herbs are traditionally used in different countries during hepatic disorders and new search is going on to isolate the active hepatoprotective principle from these herbs (Radha, 2005).

The plant Tribulus terrestris Linn (Zygophyllaceae) is a shrub, also known as puncture vine is a tap-rooted flowering plant that is widely distributed in warmer regions of the world. Tribulus terrestris Linn. Occurs throughout the India, Sri Lanka and in West Tibet. It flowers in rainy season and collected in October and November. It is an annual or biennial (Kokate, 2000). The mature plant consists of stems grown up to 2m long and the leaves consisting of 4-8 pairs of spear shaped leaflets and the fruits are like woody burr about 1 cm diameter with sharp spines to 6 mm long. Burr consists of 5 wedge shaped segments. Each segment has 2 unequal pairs of spines (Michael and Derrida). In ancient Greece and India, Tribulus Terrestris Linn. was used as a physical rejuvenation tonic.

In China, it is widely used, to this day, as a component of therapy for a number of conditions affecting the liver and kidney as well as the cardiovascular and immune systems. Tribulus terrestris Linn as a standardized pharmaceutical preparation for muscle strength and sexual potency throughout Europe, the Middle East and Asia (Michael and Derrida). It shows clinically significant effects like reduction in cholesterol level, high blood pressure and also anti-bacterial, anti-malarial and analgesic activity (Michael and Derrida).

The present study was undertaken to study the possible hepatoprotective and antioxidant role of methanol extract of fruits of Tribulus terrestris Linn against paracetamol treated hepatotoxicity in comparing with silymarin as a standard drug.
2. MATERIALS AND METHODS

Plant Material

The fruits of *Tribulus terrestris* Linn. were collected in November from Chidambaram, authenticated by a plant taxonomist, Dept. of Botany, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India. The fruits were dried under shade and mechanically powdered separately to obtain a coarse powder, which was then subjected to successive extraction in a soxhlet apparatus at 65°C for 24 hours using methanol. The resulting methanolic extract was evaporated for dryness for hepatoprotective studies.

Animals

The institutional animal ethics committee (Register No.160/1999/CPCSEA), Annamalai University, Annamalai Nagar, India approved the experimental design (Proposal No.508, dated 11-01-2008). Albino (*Wistar*) male and female rats of 140-160 g were used for the study. Animals were housed in well ventilated room (temperature 23 ± 2°C, humidity 65-70% and 12h light/dark cycle) at Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. Animals were fed with standard pellet diet and water *ad libitum*. All studies were conducted in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) norms and the National Institute of Health guidelines “Guide for the Care and Use of Laboratory Animals”.

Acute Toxicity Study

The acute toxicity study was done according to the OECD guideline 423 (Acute toxic class method). None of the rats were died at the dose of 2000 mg/kg body weight. Hence a dose of 500 mg/kg/b.wt/p.o of the crude methanolic extract of *Tribulus terrestris* L. were used for studies.

Phytochemical Screening

The methanolic extract of *Tribulus terrestris* L. of was screened for phytochemical constituents like alkaloids, carbohydrates, glycosides, flavonoids, saponins, tannins, proteins & steroids (Kokate, 2000).

Drugs and Chemicals

Silymarin, Acetaminophen was purchased from Micro labs Ltd., Bengaluru, India, Carboxy Methyl Cellulose (CMC), methanol was purchased from S.D.Fine Chemicals Ltd., Mumbai, India. The solvents/reagents obtained were used of analytical grade.

Paracetamol-Induced Liver Damage in Rats (Acute Model)

Five groups (I-V) each comprising of six male Wistar albino rats weighing in the range of 140-160 g were selected (Sini, 2006). Group I served as control and was fed orally with normal saline 1 ml/Kg daily for seven days. Groups II&IV rats were treated with METT for 7 days. Group III was treated with 1% CMC in distilled water, acts as control. Group V was fed Silymarin 25 mg/kg daily as standard for seven days. On 5th day, 2g/kg paracetamol suspended in 1% CMC was given by oral route in a dose of to all rats except rats in group I&II. The biochemical parameters were determined 18 hours after the last dose.

Biochemical Studies

Blood was obtained from all animals by puncturing retro-orbital plexus and were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 3000 rpm at 30°C for 15 min and used for the estimation of various biochemical parameters namely SGOT, SGPT, SALP, serum bilirubin and the protein content was estimated according to standard methods (Malloy and Evelyn, 1937; King and Armstrong, 1980; Reitman and Frankel, 1957).

Histopathological Studies:

After collection of blood samples the rats were sacrificed and the isolated livers were sliced into 5 mm pieces and fixed in neutral formalin (10%) solution for 3 days. Liver pieces were washed under running water for about 12 hrs. This was followed by dehydration with alcohol of increasing strength (70%, 80% and 90%) for 12 hrs each. Final dehydration was carried out using absolute alcohol with about 3 changes at 12 min interval. Cleansing was done by using xylin with changes at 15 – 20 min interval. After cleansing the pieces were subjected to paraffin infiltration in automatic tissue processing unit. The pieces were washed under running water to remove formalin completely and embedded in paraffin, sectioned, stained & mounted (Sini, 2006).

Statistical analysis

The mean ± S.E.M. was calculated for each parameter. The data obtained were subjected to the student’s ‘t’ test for unpaired data. P values of < 0.05 were considered to be statistically significant.

3. RESULTS

The methanolic extract of *Tribulus terrestris* L has shown the presence of alkaloids, carbohydrates, glycosides, flavonoids, saponins, tannins and proteins whereas steroids were absent. Table-1 represents the biochemical parameters. Administration of paracetamol (2g/kg; p.o) after, 18 hours intoxication, caused a marked elevation of enzyme levels in paracetamol treated group, in comparison with the control. There were significant
restoration of enzyme levels on administration of METT (Group IV, Table 1) and silymarin at the dose of 25 mg/kg (Group V, Table 1).

Table 1. Effect of METT on biological parameters

<table>
<thead>
<tr>
<th>Groups and Design of Treatment</th>
<th>SGOT (IU/L)</th>
<th>SGPT/ALT (IU/L)</th>
<th>ALP (IU)</th>
<th>Total Bilirubin (mg/100ml)</th>
<th>Direct Bilirubin (mg/100ml)</th>
<th>Total Protein (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I, Normal saline (ml/kg)</td>
<td>54.3±2.3</td>
<td>23.2±1.4</td>
<td>96.1±2.9</td>
<td>0.88±0.1</td>
<td>0.25±0.0</td>
<td>5.1±0.3</td>
</tr>
<tr>
<td>Group-II, METT (500 mg/kg)</td>
<td>52.1±3.4</td>
<td>23.4±1.5</td>
<td>95.2±2.8</td>
<td>0.87±0.1</td>
<td>0.25±0.0</td>
<td>5.1±0.3</td>
</tr>
<tr>
<td>Group-III, Paracetamol (25 mg/kg)</td>
<td>140.0±2.5</td>
<td>62.1±3.4</td>
<td>392.1±3.5</td>
<td>2.16±0.2</td>
<td>0.69±0.0</td>
<td>4.0±0.2</td>
</tr>
<tr>
<td>Group-IV, METT (500 mg/kg) + Paracetamol (25 mg/kg)</td>
<td>65.4±1.2</td>
<td>27.3±1.4</td>
<td>341.2±1.3</td>
<td>0.95±0.1</td>
<td>0.28±0.0</td>
<td>5.0±0.2</td>
</tr>
<tr>
<td>Group-V, Silymarin (25 mg/kg)</td>
<td>59.9±2.3</td>
<td>20.8±1.4</td>
<td>328.4±1.5</td>
<td>0.90±0.1</td>
<td>0.26±0.0</td>
<td>5.0±0.2</td>
</tr>
<tr>
<td>Group-VI, METT + Silymarin (25 mg/kg)</td>
<td>59.3±2.3</td>
<td>20.4±1.4</td>
<td>328.4±1.5</td>
<td>0.90±0.1</td>
<td>0.26±0.0</td>
<td>5.0±0.2</td>
</tr>
</tbody>
</table>

Values are mean ±S.E.M. of six animals in each group. Comparisons: a. Group II compared with group I b. Group III compared with group I c. Group IV compared with group III d. Group V compared with group III * represents the values less than P<0.05 were considered to be significant.

The histopathological profile of the rats treated with methanolic extract of Tribulus terrestris L. (Figure B) showed (H & E × 400) no visible changes confirming the safety of the extract at selected dose regimen (500 mg/kg, p.o.). Histopathological examination of liver sections of control group (Figure A) showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. In the liver sections of the rats intoxicated with acetaminophen (Figure C), there was disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis extending to mid-zone and sinusoidal haemorrhages and dilatation. There was chronic inflammatory cell infiltrate in the portal tracts.

The liver sections of the rats treated with methanolic extract of Tribulus terrestris L. and intoxicated with acetaminophen (Figure D) and rats treated with silymarin and intoxicated with acetaminophen (Figure E) showed less vacuole formation, reduced.

DISCUSSION

Paracetamol (acetaminophen), a widely used antipyretic and analgesic drug produces acute liver damage if accidental overdoses are consumed. The covalent binding of N-acetyl-p-benzoquinoneimine, 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 (Handa and Sharma, 1990; Jollow, 1973).

In the assessment of liver damage by paracetamol the determination of enzyme levels such as SGPT and SGOT is largely used. Necrosis or membrane damage releases the enzyme into circulation; therefore, it can be measured in serum. A high level of SGOT indicates liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. SGPT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, SGPT is more specific to the liver, and is thus a better parameter for detecting liver injury (Moss and Butterworth, 1974). Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Droitman and Lawhorn, 1978). Serum ALP and bilirubin level on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure (Muriel, 1992).
This present study evaluated the hepatoprotective effects of METT in paracetamol induced liver toxicity. Acute administration of paracetamol produced a marked elevation of the serum levels of SGOT, SGPT, ALP, serum bilirubin and total proteins in treated animals (Group III to V) when compared with that of the control group (Group I). Treatment with METT at a dose of 500 mg/kg significantly reduced the elevated levels of the enzymes.

Treatment with METT decreased the serum levels of SGOT, SGPT towards the respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by paracetamol. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells. Effective control of alkaline phosphatase (ALP) and bilirubin levels points towards an early improvement in the secretory mechanism of the hepatic cell.

So results of this study demonstrated that the methanol extract of *Tribulus terrestris* has significant action on paracetamol induced hepatotoxicity.

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