Hepatoprotective Activity of *Talinum portulacifolium* forsk, Extract against Paracetamol Induced Hepatic Damage in Rats

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ABSTRACT

*Talinum portulacifolium* Forsk (family: Portulacaceae) has been traditionally used in Indian medicine as a result of its curative results of hepatitis, gonorrhea and diabetes. No systemic study has been conducted on the protective effect of *Talinum portulacifolium* forsk to treat hepatic diseases. Therefore, claims can be made for the protective efficacy of *Talinum portulacifolium* forsk to treat hepatic diseases. The present study focused on investigating the role of methanolic extract of *Talinum portulacifolium* forsk (METP). METP at a dose level of 250mg/kg p.o and 500mg/kg p.o significantly produced (*p*<0.05) hepatoprotection by decreasing the level of serum Aspartate amino transferase (AST), Alanine amino transferase (ALT), alkaline phosphatase (ALP) and Total serum bilirubin (SB) However, they significantly increased the level of glutathione (GSH) in a dose dependent manner. The effects of METP were comparable to that standard drug, silymarin. Histopathological observations confirmed the beneficial role of METP against Paracetamol (PCM) - induced liver injury in rats. The result suggests that the methanolic extract of *Talinum portulacifolium* forsk possesses significant potential as a hepatoprotective agent.

Keywords: Hepatoprotective, Talinum portulacifolium forsk, Paracetamol, Lipid Peroxidation, Glutathione radicals.

1. INTRODUCTION

*Talinum portulacifolium* forsk is an annual herb which belongs to the family Portulacaceae that mainly grows in India, W. Peninsula, China and Ceylon. In India, it is found in Andhra Pradesh and Tamil Nadu. *T. Portulacifolium* forsk has been frequently used as an alternative, astringent to the bowels, worms, itching, and it is useful in gonorrhea. The juice of the leaves of the plant is used for the treatment of diabetes, cures ulcers and is traditionally used for the treatment of antioxidant. No scientific data are available to justify the traditional hepatoprotective potential of the plant.

Acetaminophen (N-acetyl-p-aminophenol, Paracetamol) is usually used as an analgesic and antipyretic drug. Extensive use of PCM for therapeutic functions leads to severe hepatic damage. Toxic doses of PCM could induce changes in the morphology and function of liver mitochondria. Formation of N-acetyl-p-benzoquinone imine (NAPQI) is responsible for liver injury through the depletion of glutathione (GSH) even as it binds to cellular proteins. PCM induced hepatotoxicity is known to involve liver cytochrome P₄₅₀ (CYPs) together CYP2E1, CYP3A4, and CYP1A2 and it also inhibits the mitochondrial oxidative phosphorylation, reduces
adenosine triphosphate (ATP) and produces selective mitochondrial oxidant stress. Cellular necrosis of the liver cells raises the lipid peroxidation and depletion of glutathione (GSH) besides elevating the serum biochemical marker levels.

The survey of literature reveals that *T. Portulacifolium* forsk is used in the traditional system of medicine as a liver tonic. However, hepatoprotective activity of *T. Portulacifolium* forsk has not been scientifically investigated. Therefore, in the present study, the hepatoprotective effect of methanolic extract of *T. Portulacifolium* forsk has been evaluated against paracetamol induced liver damage in the Wistar albino rats.

2. MATERIALS AND METHODS

2.1. Chemicals:
Paracetamol 500 mg tablets (Nirmal Prime, Mumbai, India) were used. Silymarin was purchased from Micro labs, Tamilnadu, India. Moreover, saline was purchased from GSN pharmaceutical private limited, Hyderabad, Telangana, India. The following biochemical parameters of AST, ALT, ALP and Bilirubin kits were obtained from Span Diagnostics, Surat, India. Rat’s feed was once supplied from Mahaveer Endeavors, Medipally and Hyderabad, India. All other chemicals and reagents used in the study were of analytical grade.

2.2. Plant materials:
The plant of *T. portulacifolium* forsk was collected from a mature plant during November from the wood’s territory of the Tirumala Hills, Tirupathi, Chittoor district, Andhra Pradesh (India). The plant material was taxonomically identified by Dr. K. MadhavaChetty, Department of Botany, Sri Venkateshwara University, Tirupathi, Andhra Pradesh, India and a specimen was kept in the herbarium (TP No; 2161). The plant materials were washed thoroughly to remove adhering soil and earthen matter. Later on, they were sliced into thin chips and dried in shade at room temperature and then were grounded to optimal coarse powder.

2.3. Preparation of extracts:
The powder (600 gm) was extracted at ambient temperature and at 60°C successively with (95% methanol). The solvent was changed at regular intervals of every 24 h. The alcohol from the pooled extractions were removed through distillation under reduced pressure at 50-60°C to withstand METP (50.2g). The extracts were then subjected to preliminary phytochemical investigations and subjected for hepatoprotective activity against PCM-induced liver damage.

2.4. Preliminary phytochemical studies:
The extract of *T. portulacifolium* forsk was subjected to preliminary phytochemical screening for the detection of various phytochemical constituents, such as carbohydrates, proteins, amino acids, steroids, tannins, flavonoids, terpenoids, alkaloids, mucilage, and glycosides using standard procedure. This was done to find out the nature of phytoconstituents present within them.

2.5. Experimental animals:
An experimental study was carried out using Wistar albino rats of either sex (male and female). The rats’ age was two months. Their body weights ranged from 150 to 200g. The rats were divided into 5 groups of 6 (3 male and 3 female) animals per cage were used. All animals were accepted by the ethics approval committee of the institute. (Reg No: 1648/PO/A/12/CPCSEA).

2.6. Paracetamol (PCM) induced liver toxicity:
The paracetamol (PCM) was diluted with saline (vehicle) prior to oral administration (o.p), which was done as follows: Group I: vehicle ( saline) once daily for 9 days, Group II: vehicle + PCM (1 mL/kg, p.o) once daily for 9 days, Group III: Silymarin (100 mg/kg b.wt/day, p.o) + PCM (1 mL/kg, p.o) once daily for 9 days, Group IV: AETP (250 mg/kg b.wt/day, p.o) + PCM (1 mL/kg, p.o) once daily for 9 days, and Group V: AETP (500 mg/kg
b.wt/day, p.o) + PCM (1 mL/kg, p.o) once daily for 9 days. To enhance the acute liver damage in animals of groups V, IV, III and II, food was withdrawn 12h before PCM administration. Animals were sacrificed 24h after administration of PCM. Blood samples were further collected and pooled by puncturing the retro-orbital plexus underneath using mild ether anesthesia and allowed to coagulate for 30min at 37°C. Serum was isolated by centrifugation at 2500 rpm for 15min at 35°C and further analyzed for various biochemical parameters 9-11.

2.7. Assessment of liver functions:

The hepatoprotective impact of the extract was assessed by the measure of liver, biochemical markers. Alanine Amino Transferase (ALT)14, Aspartate Amino Transferase (AST)15, Alkaline Phosphatase (ALP)16 and Total Serum Bilirubin (SB)13, Lipid Peroxidation (LPO) as Malondialdehyde (MDA)17, and Glutathione (GSH)18 were examined according to standard methods. Histopathological assessment of liver damage was done by studying Haematoxylin and Eosin (H&E) stained slides of liver tissue, including cell necrosis, fatty changes and lymphocytes19-21.

2.8. Measurement of antioxidant activity:

From all the experimental groups, livers were collected and rinsed with 0.15 M Tris-HCl (pH 7.4). A 10% w/v of liver homogenate was prepared in 0.15 M Tris-HCl buffer and processed for biochemical estimation of lipid peroxidation in the form of malondialdehyde (MDA) in the liver22. A part of homogenate, after precipitating protein, was used for the estimation of reduced glutathione (GSH)18. The rest of the homogenate was centrifuged at 1500rpm for 15min at 4°C.

2.9. Determination of lipid peroxidation in liver homogenate 22

To 0.5 mL of homogenate tissue, 0.6 mL reagent (N-methyl -2-phenylindole and acetonitrile; 3:1) 1ml BHT (butylatedhydroxytoluene) were added, mixed well and centrifuged at 3000rpm at 10min and boiled for 1h at 45°C, the tubes were then cooled at room temperature. Absorbance (UV-spectrophotometer, model UV-1601, Shimazu Corporation, Kyoto, Japan) was measured at 586 nm.

3.0. Determination of reduced glutathione (GSH)18:

Homogenate tissue (0.2mL) was mixed with 3.0mL precipitating reagent (1.67g potassium phosphate, 0.2g EDTA, 30g P-nitro benzyl chloride (PNBS) in 1 L of distilled water) and was added, mixed thoroughly and kept for 5min before centrifugation. To 2.0mL of the filtrate and absorbance were measured at 310 nm.

3.1. Statistical analysis:

The data was represented as mean ± SEM. The results were analyzed statistically by one way ANOVA test followed by Dunnet’s assessment test using orgipro (Version 7.0). The minimum level of significance was set at $P<0.05$.

3. RESULTS

Preliminary phytochemical investigation revealed the presence of flavonoids, phenols, terpenoids and steroids in methanolic extract.

3.1. Paracetamol (PCM) induced liver toxicity:

The results of hepatoprotective activity of METP on PCM treated rats are shown in Table 1. The hepatic enzymes AST, ALT, ALP and SB in serum increased significantly ($p<0.001$) in PCM treated animals compared to normal control (group-I). The METP treatments (250 and 500mg/kg) significantly increased ($p<0.05$, $p<0.01$; respectively) the levels of hepatic enzymes when compared to PCM-treated animals. Silymarin (100mg/kg) - treated animals also show significant increase ($p<0.001$) in the levels of hepatic enzymes when compared with to PCM-treated animals. There was a significant decrease ($p<0.001$) in the serum total serum albumin levels in PCM treated groups when compared to the control groups, which increased significantly ($p<0.001$) with the treatment of METP 500mg/kg and 250mg/kg, respectively.
3.2. The Effect of T. Portulacifolium forsk on antioxidant activity:

There was a significant increase in MDA content and a decrease in GSH activities of PCM intoxicated animals. Pre-treatment with silymarin (100 mg/kg b.wt/day) and T. Portulacifolium forsk (250 and 500 mg/kg p.o) significantly (p<0.05) prevented the increase in MDA levels and brought them near to normal level, whereas GSH levels increased significantly (p<0.001), thus providing protection against paracetamol toxicities. The results are presented in Table 2.

Table 1. Effect of methanolic extraction of T. Portulacifolium forsk on ALT, AST, ALP and SB in PCM induced liver toxicity in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>SB (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Vehicle (saline) 1ml/kg</td>
<td>57±1.06</td>
<td>52±2.89</td>
<td>103.33±6.52</td>
<td>0.55±0.02</td>
</tr>
<tr>
<td>Group-II</td>
<td>Control (PCM) 1 mg/kg</td>
<td>220.66±2.52\textsuperscript{a}</td>
<td>190.33±3.02\textsuperscript{a}</td>
<td>248.16±5.38\textsuperscript{a}</td>
<td>2.03±0.17\textsuperscript{a}</td>
</tr>
<tr>
<td>Group-III</td>
<td>PCM+ Silymarin 100 mg/kg</td>
<td>172±1.75\textsuperscript{***}</td>
<td>133.50±3.68\textsuperscript{***}</td>
<td>198.5±3.83\textsuperscript{***}</td>
<td>0.93±0.07\textsuperscript{***}</td>
</tr>
<tr>
<td>Group-IV</td>
<td>PCM+ METP 250 mg/kg</td>
<td>209±2.95\textsuperscript{**}</td>
<td>160.33±2.58\textsuperscript{**}</td>
<td>228.63±6.42\textsuperscript{**}</td>
<td>1.07±0.06\textsuperscript{***}</td>
</tr>
<tr>
<td>Group-V</td>
<td>PCM+ METP 500 mg/kg</td>
<td>191.83±6.17\textsuperscript{***}</td>
<td>142.16±4.66\textsuperscript{***}</td>
<td>212.16±6.73\textsuperscript{**}</td>
<td>0.99±0.156\textsuperscript{***}</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM. n =6 number of animals in each group. \textsuperscript{a}P<0.001 vs vehicle control, \textsuperscript{*}P<0.05, \textsuperscript{**}P<0.01, \textsuperscript{***} P<0.001, Compared to respective PCM treated control groups

Table 2. Effect of methanolic extraction of T. Portulacifolium forsk on lipid peroxidation (LPO), glutathione (GSH), PCM induced hepatic damage in rats

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DOSE</th>
<th>LPO (nM MDA/mg protein)</th>
<th>GSH (µg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Vehicle (saline) 1ml/kg</td>
<td>0.93±0.03</td>
<td>6.17±0.24</td>
</tr>
<tr>
<td>Group-II</td>
<td>Control (PCM) 1ml/kg</td>
<td>4.18±0.18\textsuperscript{a}</td>
<td>2.26±0.30\textsuperscript{a}</td>
</tr>
<tr>
<td>Group-III</td>
<td>PCM+ Silymarin 100 mg/kg</td>
<td>2.11±0.22\textsuperscript{***}</td>
<td>5.01±0.10\textsuperscript{***}</td>
</tr>
<tr>
<td>Group-IV</td>
<td>PCM+ METP 250 mg/kg</td>
<td>2.21±0.11\textsuperscript{***}</td>
<td>4.30±0.17\textsuperscript{***}</td>
</tr>
<tr>
<td>Group-V</td>
<td>PCM+ METP 500 mg/kg</td>
<td>2.55±0.23\textsuperscript{***}</td>
<td>4.94±0.06\textsuperscript{***}</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM. n =6 number of animals in each group. \textsuperscript{a}P<0.001 vs vehicle control, \textsuperscript{*}P<0.05, \textsuperscript{**}P<0.01, \textsuperscript{***} P<0.001, Compared to respective PCM treated control groups
3.3. Histopathological examination of rat livers:
On the ninth day, the animals were sacrificed and liver tissues were gathered. In this study, histopathological observation of liver was performed to further support the biochemical analysis evidence. The model group revealed the most severe damage of all groups. Microscopic view of liver tissue of silymarin and methanolic extracts of *T. Portulacifolium* forsk on ALT, AST, ALP and SB in PCM induced liver toxicity in rats. However, histological changes in liver tissues from groups which were treated at dose 250 and 500 mg/kg. (Fig 1A- E) were observed.

Figure 1: A) Microscopic view of liver tissue of normal rats; (B) Microscopic view of liver tissue of PCM; (C) Microscopic view of liver tissue of PCM + Silymarin; (D) Microscopic view of liver tissue of PCM + 250 mg/kg, po plant extract, (E) Microscopic view of liver tissue of PCM + 500 mg/kg, po plant extracts

Figure A: Liver tissues of control animals showing normal histology, section of normal liver tissue with portal triad showing portal vein (V), portal artery (arrow) and hepatic ducts (arrow head). Stain H and E, magnification 100X (Group I); Figure B: Liver tissue of animal treated with PCM showing necrosis (N), fatty vacuole (F) and central vein (v). Stain H and E, magnification 100x (Group II); Figure C: Liver tissue of PCM + Silymarin treated animals showing normal hepatocytes, section of normal liver tissue with portal triad showing portal vein (V), portal artery (arrow) and hepatic
ducts (arrow head). Stain H and E, magnification 100X (Group III); Figure D: Liver tissue of PCM + 250 mg/kg b.wt, po METP showing normal arrangement of hepatocytes, section of the liver tissue of PCM + 250 mg/kg b.wt, po METP treated animals showing normal arrangement of hepatocytes around the portal vein (V), absence of necrosis and moderate accumulation of fatty vacuoles (F). Stain H and E, magnification 100X (Group IV); Figure E: Liver tissue of PCM + 500 mg/kg b.wt, po CETPF showing normal arrangement of hepatocytes, Section of the liver tissue of PCM + 500 mg/kg b.wt, po METP treated animals showing normal arrangement of hepatocytes around the portal vein (V), portal artery (arrow) and hepatic ducts (arrow head). Stain H and E, magnification 100X (Group-V).

4. DISCUSSION

The *T. Portulacifolium* forsk extract has been reported to contain different types of terpenoids, based on the phytochemical screening. A number of compounds belonging to the class of polyphenol have been suggested to possess antioxidant and hepatoprotective activities. Extensive liver damage by paracetamol itself decreases its rate of metabolism and other substrates for hepatic microsomal enzymes. Interestingly, the induction of cytochrome P<sub>450</sub> or depletion of hepatic glutathione is a prerequisite for paracetamol-induced toxicity. The hepatoprotective activity of *T. Portulacifolium* forsk (500 mg/kg, p.o and 250mg/kg p.o) was compared with the activity of standard silimarin (a hundred mg/kg b.wt/day). Pretreatment of animals with methanolic extracts of *T. Portulacifolium* forsk and silimarin prevented the Paracetamol-induced rise in the serum level of transaminases and total serum bilirubin, confirming the protective effects of methanolic extract of *T. Portulacifolium* forsk against Paracetamol-induced hepatic damage.

However, there was no significant effect on the rise in serum alkaline phosphatase levels by the test extract and silimarin. Nevertheless, the Paracetamol-induced liver necrosis was once inhibited significantly using *T. portulacifolium* forsk extract, which confirms the protective action of methanolic extract of *T. Portulacifolium* forsk against experimentally-induced liver damage in rats. ALT, AST, ALP and SB are the most sensitive tests employed in the diagnosis of hepatic disease. Therefore, it can be concluded from this investigation that the extract of *T. Portulacifolium* forsk possesses hepatoprotective activity. Further, detailed studies are warranted to confirm the utility profile of this drug.

5. CONCLUSION

In conclusion, the result of this study demonstrates that *T. Portulacifolium* forsk has potent hepatoprotective action upon Paracetamol-induced hepatic damage in rats. The present study, thus, justifies the traditional use of *T. Portulacifolium* forsk in the treatment of liver diseases. It also recommends that *T. Portulacifolium* forsk warrants future detailed investigation as a promising hepatoprotective agent. However, the exact mechanism(s) and the active compound(s) involved in these effects need to be clarified in future studies.

The antidiabetic activity of *Pongamia pinnata* (Family: Leguminosae) leaf extracts was investigated in alloxan-induced diabetic albino rats. A comparison was made between the action of different extracts of *P. pinnata* and a known antidiabetic drug glibenclamide (600 μg/kg b.wt.). An oral glucose tolerance test (OGTT) was also performed in experimental diabetic rats. The petroleum ether, chloroform, alcohol and aqueous extracts of *P. pinnata* were obtained by simple maceration method and were subjected to standardization using pharmacognostical and phytochemical screening methods. Dose selection was made on the basis of acute oral toxicity study (50-5000 mg/kg b. wt.) as per OECD guidelines. *P. pinnata* ethanolic extract (PPEE) and aqueous extract (PPAE) showed significant (P < 0.001) antidiabetic activity. In alloxan-induced model, blood glucose levels of these extracts on 7th day of the study were 155.83 ± 11.211mg/dl (PPEE) and 132.00 ± 4.955mg/dl (PPAE) in comparison of diabetic control (413.50 ± 4.752mg/dl) and chloroform extract (210.83 ± 14.912mg/dl). In glucose
loaded rats, PPEE exhibited glucose level of 164.50 ± 6.350mg/dl after 30 min and 156.50 ± 4.089mg/dl after 90 min, whereas the levels in PPAE treated animals were 176 ± 3.724mg/dl after 30 min and 110.33 ± 6.687mg/dl after 90 min. These extracts also prevented body weight loss in diabetic rats. The drug has the potential to act as an antidiabetic drug.

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ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care” were followed, as well as specific national laws where applicable. All experiments have been examined and accepted by the institutional animal ethical committee (Reg No: 1648/PO/A/12/CPCSEA). All authors hereby declare that all experiments were examined and approved by the institutional ethics committee and been performed in accordance with the ethical standards.

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نشاط تكاثر الكربيوهدرات الطفيفة، مستخلص من الباراسيتامول المستوحى بالتفاقم الكلدي في الجرذان

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ملخص

تم استخدام عائلة عائلة Talinum portulacifolium Forsk Portulacaceae التي تقلد استخدمها الطبي في الهند، لعلاج التهاب الكبد والسيان و الكبد. تم إجراء دراسة تقييمية لتأثير تكرار الاضطراب الكلدي. تم استخدام عائلة عائلة Talinum portulacifolium forsk لعلاج Talinum portulacifolium forsk لعلاج الأعراض الكلدية. ركزت الدراسة الحالية على فحص دور المستخلص الميثانولوجي من Talinum portulacifolium forsk من ناحية تأثيره على مستويات AST و ALT و ALP و SB عند مستوى جرعة من METP (mg / kg b.wt / day po) 500mg و 250mg. تم تأكد أن هناك تأثيرًا ملحوظًا على مستويات GSH على مستوى مختلف من مستويات الجلود (GSH) في الجرذان. وتشير النتائج إلى أن المستخلص الميثانولوجي من Talinum portulacifolium forsk يمكن استخدامه كعلاج للثدييات، ومكافحة تأثير الالتهابات في الكبد.

الكلمات المفتاحية: نشاط تكاثر الكربيوهدرات، الباراسيتامول، تلف الكبد.

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