Evaluation of the hepatoprotective activity of *Portulaca oleracea* L. on D-galactosamine-induced hepatic injury in rats

[Véase el texto en español]

**Abstract**

The suspensions of methanol and petroleum ether extracts of entire plant of *Portulaca oleracea* in carboxymethyl cellulose (CMC) were evaluated for hepatoprotective activity in Wister albino rats by inducing hepatic injury with D-galactosamine (400 mg/kg). D-galactosamine induced hepatic damage was manifested by a significant increase in the activities of marker enzymes. Biochemical data exhibited significant hepatoprotective activity of Methanol extract of *Portulaca oleracea* at oral dose of 200 and 400 mg/kg against D-galactosamine. Silymarin was used as reference standard also exhibited significant hepatoprotective activity against D-galactosamine. The biochemical observations were supplemented with histopathological examination of rat liver sections. Phytochemical analysis of both pet ether and methanol extracts of *Portulaca oleracea* was also carried out.

**Keywords:** Portulaca oleracea; Silymarin; carboxymethyl cellulose; D-Galactosamine; hepatoprotective activity

**Resumen**

Las suspensiones de los extractos en metanol y etere de *Portulaca oleracea* (planta completa) en carboximetil celulosa (CMC) fueron evaluadas por su actividad protectora en ratas Wister albino contra el daño hepático inducido con D-Galactosamina. El daño causado por la D-Galactosamina se manifestó como un aumento significativo en la actividad de los marcadores enzimáticos. Los resultados bioquímicos mostraron un efecto protector importante del extracto metanólico de *Portulaca oleracea* contra dosis orales de 200 y 400 mg/kg de D-Galactosamina. La droga de referencia, sílimarina, también mostró un efecto protector importante. Las observaciones bioquímicas fueron suplementadas con la evaluación histopatológica de secciones de hígado. Se efectuó un análisis fitoquímico de ambos extractos.

**Palabras Clave:** Portulaca oleracea; sílimarina; carboximetil celulosa; D-Galactosamina; actividad hepatoprotectora
INTRODUCTION

Hepatitis is common disease in the world especially in the developing countries. Despite considerable progress in the treatment of liver diseases by oral hepatoprotective agents, search for newer drugs continues because the existing synthetic drugs have several limitations. Hence, there are many researchers of traditional medicines attempting to develop new drugs for hepatitis (Liu 1989).

Portulaca oleracea L. (Portulacaceae) is a warm-climate annual, it is known as Lonika in Sanskrit, Peddapayilikura in Telugu. The plant is succulent, herbaceous, erect or decumbent growing up to 30 cm height with cylindrical stem of 2-3 mm in diameter. P. oleracea used traditionally for alleviating pain and swelling (Okwuasaba et al., 1987). The entire plant is usually cut into small pieces and eaten with salt; applied topically to soothe skin (Ghazanfar 1994). It also exhibits a wide range of pharmacological effects, including antibacterial (Zhang et al., 2002), analgesic, anti-inflammatory (Chan et al., 2000), and skeletal muscle relaxant activities (Parry et al., 1987; Parry et al., 1993). A bronchodilatory effect in asthmatic patients, skeletal muscle relaxant, and antifertility effect were also reported for P. oleracea (Elkhayat et al., 2008). It is also consumed as a vegetable, widely sold in United Arab Emirates and Oman (Miller and Morris, 1988) and has been reported to be rich in α-linolenic acid and β-Carotene (Liu et al., 2000). In addition to flavonoids, coumarins (Awad, 1994) and monoterpene glycoside (Sakai et al., 1996), it also contains neither N-Trans-feruloyltyramine (Mizutani et al., 1998), dopamine, dopa, high concentrations of noradrenaline (Feng et al., 1981), ferulic acid (Chen, 2000), adenosine (Meng et al., 1981) and portulene (Elkhayat et al., 2008). This plant species is also a popular remedy in Trinidad and Tobago for urinary problems, "cooling" and high cholesterol.

The hepatoprotective activities of hydroalcoholic extract of stems and leaves of P. oleracea in rats treated with CCl₄ (Elkhayat et al., 2008) or rifampicin (Kulkarni et al., 2007) were previously reported. The present study was designed to investigate the hepatoprotective activity of petroleum ether and methanol extract of P. oleracea against D-galactosamine induced hepatotoxicity in rats.

MATERIAL AND METHODS

Plant material

The whole plant of P. oleracea was collected from surrounding Tirumala hills, Tirupathi, Andhra Pradesh in the month of November. The plant was authenticated by comparing with the specimen by Dr. K. Madhava Setty, Department of Botany, Sri Venkateshwara University, Tirupathi, Andhra Pradesh. A specimen voucher (KVCP103) has been deposited in the herbarium of department of Pharmacognosy, Sri K.V. College of Pharmacy, Chickballapur, India. The plant was cut into small pieces and coarsely powdered. The coarse powder was passed through a No. 10 mesh and used for extraction.

Preparations of plant extracts

The coarsely powdered plant material was extracted with petroleum ether and methanol by using Soxhlet apparatus sequentially. Both extracts were concentrated to dryness in vacuum using a rotary flash evaporator and yield of methanol and petroleum ether extracts were 3.13 %w/w and 1.90 %w/w. Extracts were dried in a desiccator and preserved in refrigerated condition. Both petroleum ether and methanol extracts were suspended in 0.3 % CMC just before administration to rats.

Preliminary phytochemical screening

A preliminary phytochemical screening for steroids, flavonoids, glycosides, tannins, phenolic compounds, proteins and carbohydrates were carried out using a standard procedure described by Kokate (1986).

Animals

Male albino rats of Wister strain weighing about 150-200 g of either sex were acclimatized to the experimental room temperature 23 ± 2 °C, controlled humidity conditions (50-55 %) and 12 h light and 12 h dark cycle. They were caged...
with a maximum of two animals in each polypropylene cage and were fed with standard food pellets (Kamadenu Enterprises, Bangalore) and water ad libitum. The study was conducted after obtaining ethical committee clearance from the institutional animal ethical committee of J.S.S. C P, J.S.S. College of Pharmacy, Ootacamund, Tamilnadu (India) No. IAEC/JSSCP/02/2004-2005.

Acute toxicity studies

Petroleum and methanol extracts of *P. oleracea* were studied for acute oral toxicity as per OECD (Organization for Economic Cooperation and development) guidelines No. 423, (2000). The extract was devoid of any toxicity in rats when given in dose up to 2000 mg/kg by oral route. Hence, for further studies 200-400 mg/kg doses of extract were used.

Hepatoprotective activity

Rats were randomly divided into seven groups of six animals each. Group I: Served as control and received CMC (1 ml/kg p.o.) for a period of days. Group II: Received D-galactosamine (400 mg/kg, i.p.) on 14th day. Group III: Received silymarin (25 mg/kg p.o.) for 14 days and hepatotoxicant on 14th day intraperitonially. Group IV and V: Received petroleum ether extract *P. oleracea* (200 and 400 mg/kg p.o.) for 14 days and hepatotoxicant on 14th day intraperitonially. Group VI and VII: Received methanol extract at dose *P. oleracea* (200 and 400 mg/kg p.o.) for 14 days and hepatotoxicant on 14th day intraperitonially.

After 24 h of administration galactosamine, animals were anaesthetized with pentathol sodium. Blood was collected by Sino-orbital puncture, and rats were sacrificed after the collection of blood samples and the livers were excised immediately for histopathological examination. The blood samples were allowed to clot for 30-40 min. Serum was separated by centrifugation for 15-20 min at 2000 rpm was used for estimation of various biochemical parameters (Deshpande, et al., 2003).

Biological assays

The activities of serum aspartate amino transferase (ASAT, GOT), alanine amino transferase (ALAT, GPT) (Reitaman and Frankel, 1957), alkaline phosphatase (ALP) (King and Armstrong, 1934), total bilirubin (TB) (Malloy and Evelyn, 1987), total cholesterol (TC), total protein (TP), albumin triglycerides (TGL) (Foster and Dunn, 1973) were carried out.

Histopathology

Small pieces of liver tissues were collected in 10% formal saline for proper fixation. These tissues were processed and embedded in paraffin wax. They were cut into section of 5 to 6 microns in thickness were cut and stained with hematoxylin and eosin (Luna, 1966).

Statistical analysis

The data were expressed as mean ± S.E.M. The differences were compared using one-way ANOVA followed by Dunnett’s test using PRISM software (version 4). The results were considered significant when p<0.05.

RESULTS

Petroleum ether and methanolic extracts of *P. oleracea* showed the presence of alkaloids, flavonoids, steroids, saponins, fixed oils and tannins and phenolics. Table 1 showed effect of D-galactosamine (group II) developed hepatocellular damage as evident from a significant elevation (P<0.01) in serum activities of AST, ALT, ALP, TGL, TP, Albumin and TB level when compared with control. The pet ether and methanolic extract of *P. oleracea* showed significant restoration of the altered biochemical parameters (p<0.01 and p<0.05) at both the dose levels (200 and 400 mg/kg) when compared to d-galactosamine treated group. Silymarin also showed similar effect.
Figure 1. Histopathological observations.

Group 1. Rats treated with CMC solvent showing structure of normal liver (H&E 400x).

Group 2. Rats treated with D-galactosamine showing severe periportal inflammation necrosis of zone 1 hepatocytes and bile duct hyperplasia (H&E 400x).

Group 3. Rat treated with D-galactosamine Silymarin showing focal necrosis and periportal inflammation (H&E 400x).

Group 4. Rat treated with D-galactosamine and 200 mg/kg of pet ether extract of *P. oleracea* showing foamy degeneration of hepatocytes (H&E 400x).

Group 5. Rat treated with D-galactosamine and 200 mg of methanol extract of *P. oleracea* showing foamy degeneration of hepatocytes and regenerative activity (H&E 400x).

Group 6. Rat treated with D-galactosamine and 400 mg of pet ether extract of *P. oleracea* showing foamy degeneration of hepatocytes (H&E 400x).

Group 7. Rat treated with D-galactosamine and 400 mg of methanol extract of *P. oleracea* showing foamy degeneration of hepatocytes (H&E 400x).
Galactosamine administration in rats disrupts the membrane permeability of the plasma membrane causing leakage of the enzymes from the cell, which leads to elevation in levels of serum enzymes (Mitra et al., 2000). Elevated serum enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in liver (Drotman and Lawhorn, 1978). Hence significant rise in the transaminase levels could be taken as an index of liver damage. Further, intense galactosamination of membrane structure is thought to be responsible for loss of activity of ionic pumps. The impairment in the calcium pump, with consequent increase in the intracellular calcium is considered to be responsible for cell death (Tsai et al., 1997). The rise in ALAT activity is almost always due to hepatocellular damage and is usually accompanied by rise in ASAT (Rao et al., 1989). An increase in ALP reflects the pathological alteration in biliary flow (Plaa and Hewitt, 1989). Determination of serum bilirubin represents an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary disease and server disturbance of hepatocellular function.

Histology of the liver sections of normal control animals (Group I) showed normal liver architecture. The liver section of galactosamine treated (Group II) showed hepatic cells with severe toxicity characterized by inflammatory cell collection, scattered inflammation across liver parenchyma, focal necrosis and swelling up of vascular endothelial cells. Petroleum ether and methanol extracts of *P. oleracea* appeared to significantly prevent the galactosamine toxicity as revealed by the hepatic cells with well-preserved cellular architecture (Group III-VII).

**DISCUSSION**

Galactosamine induced experimental model system in rats is recognized to be much like viral hepatitis in humans from both morphological and functional points of view (Keppler et al., 1968). Galactosamine has great liver specificity because hepatocytes have high levels of galaktokinase and galactose-1-uridyltransferase. Galactosamine does not affect other organs (Maley et al., 1986 and Keppler et al., 1970). Galactosamine causes hepatic injury with spotty hepatocyte necrosis and marked portal and parenchymal infiltration (Keppler and Decker, 1969). Galactosamine also causes depletion of uridine diphosphate (UDP) by increasing the formation of UDP-sugar derivatives, which results in inhibition of RNA and protein synthesis leading to cell membrane deterioration (Decker et al., 1973; El-Mofty et al., 1975).

### Table 1. Effect of the petroleum ether and methanol extracts of *P. oleracea* on D-galactosaminic-induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvent</th>
<th>D(+)galN</th>
<th>Silymarin</th>
<th>Pet ether extract</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td></td>
<td>400</td>
<td>25</td>
<td>200</td>
<td>400</td>
</tr>
<tr>
<td>TB (mg/dl)</td>
<td>-</td>
<td>0.78±0.10</td>
<td>4.03±0.07</td>
<td>1.62±0.09**</td>
<td>3.43±0.19**</td>
</tr>
<tr>
<td>ASAT (U/L)</td>
<td>28.33±1.48</td>
<td>73.33±3.40</td>
<td>31.83±2.02</td>
<td>68.83±2.81**</td>
<td>75.83±2.77**</td>
</tr>
<tr>
<td>ALAT (U/L)</td>
<td>18.83±0.60</td>
<td>84.83±3.44</td>
<td>23.17±0.95</td>
<td>74.67±1.89**</td>
<td>76.17±2.46**</td>
</tr>
<tr>
<td>TGL (mg/dl)</td>
<td>79.83±0.87</td>
<td>25.67±1.76</td>
<td>71.17±2.27*</td>
<td>31.5±2.14**</td>
<td>36.67±0.88**</td>
</tr>
<tr>
<td>TC (g/dl)</td>
<td>48.0±1.21</td>
<td>174±2.96</td>
<td>72.17±2.75**</td>
<td>172.83±4.21**</td>
<td>167.67±6.22**</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>5.18±0.11</td>
<td>3.55±0.16</td>
<td>1.42±0.14**</td>
<td>3.23±0.15**</td>
<td>2.30±6.22**</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>9.90±0.15</td>
<td>5.35±0.40</td>
<td>8.0±0.12**</td>
<td>4.33±0.23**</td>
<td>4.38±0.31**</td>
</tr>
<tr>
<td>ALP</td>
<td>197.68±6.82</td>
<td>373.33±17.7</td>
<td>215.17±6.46**</td>
<td>447.50±18.22**</td>
<td>415.83±4.32**</td>
</tr>
</tbody>
</table>

Mean ± S.E.M.; (n=6); *P<0.05, **P<0.01 as compared with control (CMC) Dunnett’s test. TB: Total bilirubin. ASAT: asparatate amino transferase. ALAT: alanine amino transferase. TGL: triglycerides. TC: Total cholesterol. TP: Total proteins. ALP: alkaline phosphatase.
Prabhakarn et al. Hepatoprotective activity of *P. oleracea* on D-galactosamine-induced hepatic injury in rats

(Martin and Friedman, 1992). Increased levels of bilirubin in this study are in agreement with previous reports showing that d-GalN induced hepatitis is characterized by increased levels of bilirubin in serum (Sree Ramamurthy and Srinivasan, 1993; Maezona et al., 1996). The extract-mediated suppression of the increased bilirubin level suggests the possibility of the extract being able to stabilize biliary dysfunction. Hepatocellular damage due to alcohol, virus and drug induced hepatitis causes a modest hypertriglyceridemia (Glickman and Sebesin, 1982) which is due to the biochemical changes inferring with the transport of triglycerides out of liver. Our study also showed an increased accumulation of triglycerides in D (+) galactosamine induced rats, which is in agreement with previous reports (Koff et al., 1971; Cartwright et al., 1982). Pre treatment with methanol extract of *P. oleracea* and Silymarin for 14 days protected the rat livers from D-galactosamine induced histopathological changes.

**CONCLUSION**

The results suggest that extracts from *P. oleracea* possess significant protection against galactosamine induced hepatotoxicity in rats.

**ACKNOWLEDGEMENTS**

The authors wish to thank the management, Sri K.V. College of Pharmacy, Sri K.V. Naveen Kiran, Chairman, K.V. and Panchgiri Trust, Chickballapur, Karnataka (India) for providing necessary facilities and support for the completion of this work.

**REFERENCES**


Kulkarni AS, Siraskar BD, Deshpande AD, Kulkarni AV, Dhone SM, Bingi. 2007. Study of hepatoprotective


