PHYTOCHEMICAL AND PHARMACOLOGICAL ANALYSIS OF ETHANOLIC EXTRACTS OF *PROSOPIS JULIFLORA* (SW.)

D. S. Prabha¹, P. Mani*², P. Malliga³ and H. U. Dahms⁴

¹Dept of Microbiology, Annai College of Arts & Science, Kumbakonam, Tamilnadu, India.
²Dept of Biotechnology, Annai College of Arts & Science, Kumbakonam, Tamilnadu, India.
³Department of Marine Biotechnology, National Facility for Marine Cyanobacteria, Bharathidasan University, Tiruchirapalli, 620024, India.
⁴Kaohsiung Medical University, Department of Biomedical Science and Environmental Biology Kaohsiung 80708, Taiwan R.O.C.

**ABSTRACT**

*Prosopis juliflora* is a commercially important plant genus, which has been used since ancient times, particularly for medicinal purposes. Traditionally, Paste, gum, and smoke from woody stems, leaves and pods are applied for anticancer, antidiabetic, anti-inflammatory, and antimicrobial purposes. This study was carried out to investigate the acute and subacute oral toxicity of the ethanolic extract of *Prosopis juliflora* on wistar rats. In this study preliminary phytochemical analysis showed that there are some plant chemicals constituents present in the *P. juliflora* extract such as alkaloids, flavanoids, terpenoids and steroids. GC-MS result revealed that more than 15 compounds present in the *P. juliflora*. In an acute toxicity study *P. juliflora* extract was administered orally at doses ranging from 50-500 mg kg⁻¹ and the animals were observed for any toxic symptoms for 72 hrs. The results did not show toxic symptom below a dose level 200 mg kg⁻¹. In a subacute toxicity study ethanolic extracts of *P. juliflora* were tested at a dose of 200 mg kg⁻¹ orally once daily for 30 days. On 31ˢᵗ day the animals were sacrificed and the blood and serum samples were analyzed for various biochemical parameters. The results of this subacute toxicity study in experimental animals did not show any changes in hematological, biochemical, renal and liver function parameters when compared to control.
animals. From these results it is evident that ethanolic extracts of \textit{P. juliflora} were non toxic and can be further used for long term \textit{in vivo} studies for pharmacological effects.

**KEYWORDS:** \textit{Prosopis juliflora}, acute toxicity, subacute toxicity, \textit{Rattus norvegicus}.

**INTRODUCTION**

The uses of medicinal plants for the treatment of various diseases are actively practiced from ancient periods until nowadays where herbal drugs are competing with synthetic drugs. There is a growing interest in herbal remedies due to the side effects associated with the existing therapeutic agents.\cite{1} Systemic investigations on medicinal plants should, therefore, be carried out to identify new bioactive substances, which could be used as effective therapeutic agents. \textit{Prosopis juliflora} (Sw.) (Leguminosae) commonly known as mesquite is a shrub or small tree native to Mexico, South America and the Caribbean.\cite{2} \textit{Prosopis} comprises of 44 species distributed mainly in arid, semi-arid, tropical and subtropical countries.\cite{3} Many plants of the genus \textit{Prosopis} are known to have medicinal properties and are used in folk medicine as astringents, in rheumatism and as remedies against scorpion stings and snake bites.\cite{4} The members of the \textit{Prosopis} species are rich in phenols, piperidine alkaloids, flavonol glycosides, hydroxycinnamic acids, juliprosopine and mesquitol.\cite{5, 6} Some \textit{Prosopis} species have antidermatophytic, antibacterial, antifungal, hemolytic, anti-inflammatory, antihypercholesterolemic, antitumour and antioxidant properties. As \textit{P. juliflora} has traditional medicinal values which have not been fully realized, this study was carried out to investigate the acute and subacute toxicity of the ethanolic extract of \textit{P. juliflora} wood to an experimental animal model as an initial step for its subsequent pharmacological screening.

**MATERIALS AND METHODS**

**Plant material**

Woody stems were collected from the tree \textit{Prosopis juliflora} found in Bharathidasan University campus, Tiruchirappalli, India. The finely chopped wood was dried under sunlight and ground to powder in a ball mill and passed through a 115-mesh sieve and dried at 60° C before extraction.

**Wood Extraction**

Soxhlet extraction was done where 10 g of the powdered sample was extracted with 180 ml of ethanol for 15 hours at a rate of 10 to 12 cycles per hour.\cite{13} After extraction, the solvent
was evaporated under reduced pressure in a Buchi rotavapor and the crude extract was dried under vacuum in a desiccator over P₂O₅.

**Phytochemical analysis**

Preliminary phytochemical tests were carried out on the ethanolic extract of *P. juliflora* wood using standard procedures to identify the constituents as described by TREASE and EVANS (1983) and HARBORNE (1973).

**Gas Chromatography-Mass spectrometry (GC-MS) analysis**

The GC-MS analysis was carried out using a Clarus 500 Perkin-Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised up to 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

**Experimental Animals**

Male albino rats of the Wistar strain (*Rattus norvegicus*) (150-180 g) were maintained under standard laboratory conditions with a standard pellet diet (Sai Durga Feeds, Bangalore) and water *ad libitum*. They were selected for the experiments by random sampling.

**Acute toxicity study**

An acute toxicity study was performed according to OECD guidelines. All animal experiments were carried out according to the guidelines of the Institutional Animal Ethics Committee (NCP/IAEC/BU-01/2009). Before experimentation, the animals were fasted for four hours with free access to water only. Twenty five male rats were divided into five groups, containing 5 rats each and tested up to 72 hrs. Group I received normal saline orally
(control group). Group II, III, IV and V received 50, 100, 200 and 500 mg kg\(^{-1}\) of *P. juliflora* wood extract for the assessment of mortality rate.

**Subacute toxicity study**
A subacute toxicity study was carried out with the selected dose on the basis of acute toxicity studies. The LD\(_{50}\) of the extract was found to be 200 mg kg\(^{-1}\) body weight. The animals were separated into two groups of 6 animals each. Group I received normal saline orally (control group). Group II received *P. juliflora* with a dose of 200 mg kg\(^{-1}\) body weight for 30 days. At the end of the experiment, blood samples were collected by retro orbital bleeding for biochemical analysis.

**Haematological and biochemical analysis**
The haematological and biochemical analysis were measured using a fully automated analyzer (Erba Mannheim EM 360 clinical chemistry analyser, Mannheim, Germany) using Commercial Erba kits (Erba Diagnostics Mannheim, Germany). Blood samples collected under 10% EDTA were examined for Haemoglobin (Hb), Erythrocyte Sedimentation Rate (ESR), Packed Cell Volume (PCV), Red Blood Cells (RBC), White Blood Cells (WBC), Neutrophil, Lymphocyte and Eosinophil. The separated serum was analyzed for Protein, Albumin, Globulin, Cholesterol, Triglycerides (TGL), Urea, Uric acid, Creatinine, Total bilirubin, Indirect bilirubin, Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline Phosphatase (ALP).

**Statistical analysis**
The data were presented as means ± SE. Results were analyzed using one-way ANOVA and considered as being significant at p<0.05.

**RESULTS AND DISCUSSION**

**Phytochemical**
Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value. The extracts of *P. juliflora* revealed the presence of plants secondary metabolites in the form of phytochemicals, vitamins and vital minerals. The biologically active chemical substances have curative properties. In this study preliminary phytochemical analysis showed that there are some plant chemicals constituents present in the *P. juliflora* extract such as alkaloids, flavonoids, terpenoids and steroids. GC-MS result revealed that more than 15 compounds present in the *P. juliflora*.(Table.1)
Acute toxicity

In the acute toxicity study, ethanolic extracts up to the dose of 200 mg kg$^{-1}$ of body weight did not exhibit any toxic symptoms. As the above mentioned dose was well tolerated by the experimental animal without any behavioral changes during long term treatment, further studies were carried out with a dose of 200 mg kg$^{-1}$ of body weight. A similar acute toxicity study revealed that the methanolic extract of *P. juliflora* bark was safe up to a dose level of 400 mg kg$^{-1}$ of body weight.$^{[14]}$ In a preliminary study done by Quintas-Junior$^{[15]}$, the total alkaloid fraction of the pods of *P. juliflora* showed an acute toxicity (LD$_{50}$) of 10.3 mg kg$^{-1}$ intraperitonially and 637 mg kg$^{-1}$ orally. Lydia$^{[16]}$ reported that the oral median lethal dose of the methanolic extract of *Prosopis africana* was 3.808 g kg$^{-1}$ in mice and > 5 g kg$^{-1}$ in rats. This study also supports the folkloric claim of the analgesic and anti-inflammatory effects of *P. juliflora*.

Subacute toxicity

In the subacute toxicity study, the ethanolic extract of *P. juliflora* treated group did not show any significant changes in haematological parameters like haemoglobin, erythrocyte sedimentation rate, packed cell volume, red blood cell count, total and differential white blood cell count when compared to the control group (Table 2). This indicates that the ethanolic extract of *P. juliflora* may not be toxic and this may be due to the antioxidant property of the phenols and flavonoids present in the extract.$^{[6, 17]}$ Similarly, administration of *Artemisia judaica* (0.5 and 1 g kg$^{-1}$ body weight) containing flavonoids as the major component in diabetics induced rats did not show significant effects on the hemoglobin content, packed cell volume, erythrocytic count, total leucocytic count and differential leucocytic count in rats throughout the experimental period.$^{[18]}$ The methanolic extract of *Arthospira platensis* is known to be rich in antioxidants such as phenolic acids, α-tocopherol and β-carotene. It was examined for subchronic toxicity at doses of 6, 12 and 24 mg kg$^{-1}$ body weight daily for 12 weeks, showing normal white blood cells counts in all treated groups similar to the control. Also, no significant changes were observed in differential counts.$^{[19]}$ It is reported that *Artemisia afra* did not cause any significant changes in hematological parameters in rats after oral administration of the aqueous plant extract for a period of 3 months.$^{[20]}$

There was no significant change in serum protein, albumin, globulin of experimental rats when compared to control groups (Table 3). The cholesterol and triglyceride levels were
found to be significantly lowered (p < 0.05) in rats treated with ethanolic extract of *P. juliflora* when compared to control rats (Table 2) which may be due to hypocholesteremic effects of the administered extract. Narasimhacharya\[11\] reported that administration of *P. juliflora* leaf powder in the diet to hypercholesterolemic rats for a four week period resulted in a significantly lowered total lipid plasma content, total cholesterol, triglycerides, LDL-C, VLDL-C and atherogenic index accompanied by a significant increase in HDL-C levels. Barros\[21\] reported that the ethanolic root extract of *Pothomorphe umbellata* L. contains 6.5% of 4-nerolidilcatecol, a phenolic compound with high antioxidant activity. When administered at 500 mg kg\(^{-1}\) body weight for a period of 40 days, no significant variation in hematological and biochemical parameters of treated rats were shown. Phenolic compounds extracted from the leaves of *Myrtus communis* when administered to streptozotocin induced diabetic rats at 800 mg kg\(^{-1}\) body weight recorded a normal level of total proteins and showed a drastic decrease in albumin and a corresponding increase in globulin when compared to normal rats which indicates hepatic damage.\[22\] This may be due to a high consumption of phenolic compounds that reduced cholesterol levels. This result concurred with an early study made by Fahim\[22\] reporting phenolic compounds extracted from the leaves of *Myrtus communis* when administered at 800 mg kg\(^{-1}\) body weight showed a progressive decline in cholesterol levels towards normal while diabetic rats treated with 400 mg kg\(^{-1}\) showed only a moderate decline. As flavonoids are known for their diverse biological activities ethanolic extracts of *Clerodendron phlomoidis* when administered orally result in a significant reduction of serum lipid in rats with hyperlipidemia.\[23\] Reports showed that phenolic compounds, propenylbenzenes and some alkaloids decrease the cholesterol and triglycerides in rats.\[24, 25\] Antia and Okokon\[26\] reported the effect of leaf extract of *Catharanthus roseus* Linn. when administered orally at 0.1, 0.5 and 1.0 mg kg\(^{-1}\) d\(^{-1}\) for seven consecutive days, showing a significant decrease in total serum cholesterol, total triglycerides, LDL-cholesterol and VLDL-cholesterol of rats.

The normal level of urea, uric acid and creatinine indicate that the ethanolic extract of *P. juliflora* did not interfere with the renal function (Table 4). Also, there was no significant change in total and indirect bilirubin, SGPT, SGOT and ALP in experimental groups when compared to the control group (Table 3). Phenolic compounds alone, therefore did not exhibit any toxic effects in the tested animals. Supporting evidence showed that polyphenolic extracts have protective effects against hepatic cell injury induced by thioacetamide.\[27, 28\] Bairwa\[29\] reported that administration of the ethyl acetate fraction of stem bark of *Ceiba*
*pentandra* (400 mg kg\(^{-1}\)) containing phenolic compounds possesses a hepatoprotective potential in hepatotoxicity induced rats by paracetamol (3 g kg\(^{-1}\)), which showed a significant reduction in the serum enzymes SGOT, ALP and the total bilirubin content. Similarly, *Anogeissus latifolia* administered at 300 mg kg\(^{-1}\) showed a significant reduction in the above enzymes.\(^{[30]}\) Therefore, rutin, quercetin and other antioxidants in plant extract may be the contributing factors towards its hepatoprotective activity.

**Table 1:** Qualitative analysis of phytochemicals present in the ethanol extracts of *P. juliflora*.

<table>
<thead>
<tr>
<th>Test analyzed</th>
<th><em>P. juliflora</em></th>
<th>Color development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+ve</td>
<td>Reddish brown precipitate</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+ve</td>
<td>Yellow color</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+ve</td>
<td>Reddish brown precipitate</td>
</tr>
<tr>
<td>Saponins</td>
<td>-ve</td>
<td>Froth formation</td>
</tr>
<tr>
<td>Steroids</td>
<td>+ve</td>
<td>Reddish brown color</td>
</tr>
</tbody>
</table>

**Table 2:** Effect of the ethanolic extract of *P. juliflora* on haematological parameters of rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
<th>Hb (g dl(^{-1}))</th>
<th>ESR (mm hr(^{-1}))</th>
<th>PCV (%)</th>
<th>RBC (mm(^3))</th>
<th>WBC (mm(^3))</th>
<th>N (%)</th>
<th>L (%)</th>
<th>E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>17.2±1.1</td>
<td>7±0.9</td>
<td>42±1.5</td>
<td>5.7±0.2</td>
<td>6.9±0.7</td>
<td>28±1.2</td>
<td>60±2.3</td>
<td>3±0.6</td>
</tr>
<tr>
<td><em>P. juliflora</em></td>
<td></td>
<td>14.8±0.9</td>
<td>9±0.8</td>
<td>44±2.3</td>
<td>5.4±1.5</td>
<td>7.1±3.1</td>
<td>36±2.6</td>
<td>61±1.5</td>
<td>2±0.2</td>
</tr>
</tbody>
</table>

Data are the mean ± S. E. of 6 animals (One-way ANOVA). Hb- Hemoglobin, ESR- Erythrocyte Sedimentation Rate, PCV- Packed Cell Volume, RBC- Red Blood Cells, WBC- White Blood Cells, N- Neutrophil, L- Lymphocyte, E- Eosinophil.

**Table 3:** Effect of the ethanolic extract of *P. juliflora* on biochemical parameters of rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
<th>Protein (g dl(^{-1}))</th>
<th>Albumin (g dl(^{-1}))</th>
<th>Globulin (g dl(^{-1}))</th>
<th>Cholesterol (mg dl(^{-1}))</th>
<th>Triglyceride (mg dl(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>7.1±0.3</td>
<td>4.7±0.3</td>
<td>3.5±0.2</td>
<td>136.2±4.8</td>
<td>117.5±2.6</td>
</tr>
<tr>
<td><em>P. juliflora</em></td>
<td></td>
<td>6.9±0.2</td>
<td>4.5±0.2</td>
<td>3.2±0.1</td>
<td>97.4*±5.1</td>
<td>83.7*±1.4</td>
</tr>
</tbody>
</table>

Data are the mean ± S. E. of 6 animals (One-way ANOVA). Values are statistically significant at *p < 0.05.
Table 4: Effect of the ethanolic extract of *P. juliflora* on kidney and liver function parameters of rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
<th>Parameter</th>
<th>Parameter</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urea (mg dL⁻¹)</td>
<td>Uric acid (mg dL⁻¹)</td>
<td>Creatinine (mg dL⁻¹)</td>
<td>Total Bilirubin (mg dL⁻¹)</td>
</tr>
<tr>
<td>Control</td>
<td>26.5±1.7</td>
<td>6.4±0.4</td>
<td>0.7±0.04</td>
<td>0.9±0.05</td>
</tr>
<tr>
<td><em>P. juliflora</em></td>
<td>22.5±1.2</td>
<td>5.8±0.7</td>
<td>0.5±0.07</td>
<td>0.9±0.03</td>
</tr>
</tbody>
</table>

Data are the mean ± S. E. of 6 animals (One-way ANOVA). SGPT- Serum Glutamic Pyruvic Transaminase, SGOT- Serum Glutamic Oxaloacetic Transaminase, ALP- Alkaline Phosphatase.

CONCLUSION

Several medicinal plants have been used by humans without a precise knowledge of their potential toxicity. *P. juliflora* is one of such plants used for the treatment of various diseases in traditional medicine. The growing interest in bioactive products from natural sources demands toxicity risk assessments, such as for *P. juliflora* as a prerequisite for its pharmacological use. The present findings suggest that 200 mg kg⁻¹ *P. juliflora* extract is nontoxic for the fact that no marked changes in hematological, biochemical parameters were observed. Such relatively high nontoxic dose allows for further long term experimental studies. This may reveal several pharmacologically active compounds from *P. juliflora* that can be used in various therapeutic applications.

ACKNOWLEDGEMENT

The facilities availed from the Department of Marine Biotechnology, National Facility for Marine Cyanobacteria, Bharathidasan University, Tiruchirappalli, Tamilnadu and Nandha College of Pharmacy, Erode, India are gratefully acknowledged.

REFERENCES


