Antioxidant and Hepatoprotective Potential of Stem Methanolic Extract of Justicia gendarussa Burm

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Abstract: In vitro antioxidant activity of stem extracts of Justicia gendarussa Burm along with in vivo hepatoprotective activity of methanolic fraction was carried out to ascertain the folkloric claim of its hepatoprotective activity. The crude methanolic extract was prepared by soxhlet extraction and fractionated into pet ether, chloroform and methanol fractions. The marc remained was further extracted with double distilled water by refluxation in water bath. Preliminary phytochemical tests, total phenolic and flavonoid content present in each fraction/extract was determined. All fractions and aqueous extract were evaluated for their antioxidant activity using DPPH free radical scavenging activity, hydrogen peroxide scavenging activity, reduction of ferric ion in presence and absence of EDTA. The methanolic fraction was further studied for its in vivo hepatoprotective activity using CCl₄ induced hepatotoxicity in albino rats. The various biochemical parameters were evaluated to asses its hepatoprotective activity. Methanolic fraction has more phenolic/flavonoid content and shows good antioxidant activity. The methanolic fraction has a good hepatoprotective activity at dose of 300 mg kg⁻¹ b.wt. in albino rats. Interestingly its hepatoprotective activity decreases as the dose increases. Stem extract of JG has moderate hepatoprotective activity; it may be due to its total phenolic and flavonoid contents. These findings substantiate the ancient literature about its reports as hepatoprotective herb and inspires for further extensive study regarding its exact hepatoprotective activity.

Key words: Justicia gendarussa, hepatoprotective, CCl₄, methanolic

INTRODUCTION

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, through relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine.

Justicia gendarussa Burm (JG) belonging to family Acanthaceae and it is considered as native of China. It is frequently grown in Indian gardens as hedge or border plant; it is sometimes found as an escape. It is propagated by cuttings and grows quickly. It is hardy, withstands heavy rainfall and thrives in shade. The plant is used in conditions such as inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases and fever in Ayurveda, an Indian system of medicine. A decoction of the root boiled in milk is given in rheumatism, dysentery and jaundice (Kirthikar and Basu, 2005). The stems are reported to contain a bitter and slightly toxic alkaloid. A decoction of alcoholic extract of the roots produced slight paralysis in rats in doses of 1-2 g kg⁻¹ b.wt. and in doses of 10-20 g kg⁻¹ it is antipyretic and depressant producing violent diarrhoea eventually death. Decoction of leaves is used to treat chronic rheumatism. It is reported that the flowering head along with the portion of stem is used as demulcent and astringent (Anonymous, 1959; Chopra et al., 1956; Kirthikar and Basu, 2005). It is also reported to contain β-sitosterol, friedelien, lupool and four simple 0-substituted aromatic amines (Chakravarty et al., 1982). Recently, plant extracts were reported for their anti-inflammatory and antioxidant (Devprakash, 2000), reverse transcriptase inhibitory activity (Woradulayapinj et al., 2005), analgesic activity (Ratnasooriya et al., 2007), antioxidant potential of aqueous ethanolic extract of JG (Mruthunjaya and Hukkeri, 2007).

Also the plant is used in treatment of jaundice in locals in the state of Gujarat, India. The hepatoprotective activity of Justicia gendarussa has not been evaluated scientifically to substantiate its ancient literature regarding its usefulness as hepatoprotective herb (Pandey et al., 2005). So, this study was conceptualized to evaluate its hepatoprotective property. Antioxidant and free radical scavenging activities of herbs are reported to
be responsible for the hepatoprotective activity. JG was reported to possess antioxidant activity (Mrunalini and Hukkeri, 2007). The hepatoprotective activity of JG may be due to its free radical scavenging activity. So in the present study extracts of JG were subjected for in vitro antioxidant and free radical scavenging activities prior to the in vivo hepatoprotective activity.

MATERIALS AND METHODS

Plant material: Stem samples were collected from the Veer Nariman South Gujarat University Campus, Surat, India, in the month of September 2007 and sample was identified and authenticated by Dr. Minu Parabia, Professor and Head, Department of Bioscience. A specimen sample (KLKJG01) was deposited in the Department of Bioscience.

Preparation of the extracts: Five hundred gram of Shade dried stems powder of the plant was extracted with 5 L of methanol using Soxhlet method till the exhaustion for about 24 h (ME=13.82%). Marc obtained was further extracted with pure Doubled Distilled (DD) water using water bath by reflux (WE=1.73 %). Both the extracts were concentrated under vacum to get the residues. The methanolic extract was further fractionated to petroleum ether (PEF-8.9%), chloroform (ChF-7.8%) and methanol (MP-83.3%).

In vitro antioxidant activities: The extracts were subjected for the detailed preliminary phytochemical studies to know the phytochemical nature (Evans, 2005; Finar, 2006; Kokate et al., 1996; Onwukaeme et al., 2007).

Determination of total phenolics: The total phenolic content of all extracts were determined by using Folin-Ciocalteu's assay (Marinova et al., 2005). An aliquot (0.4 mL) of extract or standard solution of gallic acid (1, 5, 10, 15 and 20 µg mL⁻¹) was added to 10 mL volumetric flask, containing 3.6 mL of doubled distilled water. Reagent blank was prepared using doubled distilled water. Folin Ciocalteu’s Phenol reagent (0.4 mL) was added to the mixture and shaken. After 5 min, 4 mL of 7% Na₂CO₃ solution was added to the mixture. The solution was diluted to volume (10 mL) with DD water and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with an UV-Visible Spectrophotometer Shimadzu 1700. Total phenolic content of various extracts were expressed as mg Gallic Acid Equivalent (GAE)/100 g of extract. All samples were analyzed in triplicate.

Estimation of total flavonoids: Total flavonoid content present in the various extracts were determined by the aluminium chloride calorimetric assay (Marinova et al., 2005). An aliquot (0.5 mL) of extract or standard solution (quercetin) was added to test tube containing 2 mL of DD water and 0.15 mL of 5% Sodium nitrite. After 5 min, 0.15 mL of 10% aluminium chloride solution was added. At 6th min, 2 mL of 1 M Sodium hydroxide was added and the total volume was made to 5 mL with DD water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content of the extracts was expressed as mg of quercetin equivalent to 1 g of the extract. All experiments were done in triplicate and total flavonoid content was given in Mean±SD.

Free radical scavenging activity by DPPH method: Free radical scavenging potentials of the extracts were tested against a methanolic solution of α, α-diphenyl-β-pieryl hydrazyl using the method of Choi et al. (2002). 2.5 mL of sample solution of different concentrations or standards were mixed with methanolic solution of DPPH (0.3 mM) and allowed to react at room temperature. Ascorbic acid, BHT and Gallic acid were used as standards. The absorbance was read after 30 min at 518 nm against methanol as blank. Percentage Radical Scavenging activity was calculated by the formula:

\[
\text{Scavenging capacity (\%)} = \frac{100 - (\text{Ab. of sample}-\text{Ab. of blank})}{\text{Ab. of control}} \times 100
\]

Reduction of ferric ions: The reaction mixture containing o-phenanthroline (0.5 mg), ferric chloride (0.2 mM) and test compounds (extracts/standard) dissolved in 0.2 mL ethanol in a final volume of 5 mL was incubated for 10 min at ambient temperature. The absorbance at 510 nm was measured. In another experiment, sodium dithionite (0.3 mM) was added instead of the test compound and the absorbance obtained was taken as equivalent to 100% reduction of all the ferric ions present (Rajakumar and Rao, 1993). All experiments were carried out in triplicate and % ferric ion reduction activities of various extracts were reported as Mean±SD.

The reduction of ferric ion in presence of EDTA also determined as the method of Mruthunjaya and Hukkeri (2007).

Hydrogen peroxide scavenging activity: Hydrogen peroxide scavenging ability of extracts was determined by simple UV spectroscopic method. All extracts were taken at the different concentration of 50 to 200 µg mL⁻¹. Volume adjusted to 3 mL with phosphate buffer and 1 mL
of 30 mM H$_2$O$_2$ was added. After 10 min, the absorbance value of the reaction mixture was recorded at 230 nm. Blank solution was containing the phosphate buffer without H$_2$O$_2$ (Kaur et al., 2006). The percentage of scavenging activity was calculated by using below formula:

\[
\text{Percentage scavenged} \ [\text{H}_2\text{O}_2] = \frac{A_0 - A_t}{A_0} \times 100
\]

where, $A_0$ is the absorbance of the control and $A_t$ is the absorbance of the sample.

**Animal studies**

**Experimental animals:** Forty six Wistar strain rats of either sex weighing 150-220 g were procured from the JSS Medical College, Central Animal Facility Centre, Mysore, Karnataka, India. The animals were housed in polypropylene cages and maintained in controlled temperature (27±2°C) and light cycle (12 h light and 12 h dark). They were fed with rat feed (Hindustan Animal Feeds) and water ad libitum. The animals were acclimatized with the laboratory conditions before the commencement of the experiment. Ethical clearance for the use of animals was obtained from the IAEC of JSS College of Pharmacy, Mysore, India. (Proposal No. 029/2008, dated 25th July 2008).

**Acute toxicity studies:** Acute toxicity study of methanolic fraction of JG was done according to the acute toxic classic method (OECD guideline 425, 2001) using albino female rats. The animals were kept fasting for overnight providing only water, after which the extract was administered orally for one animal at the limit dose of 2000 mg kg$^{-1}$ and observed for 14 days (with special attention for the first 4 h of administration followed by the next 20 h). If the animal dies, the limit test was terminated and main test was conducted. If the animal survives, four additional animals were dosed sequentially so that five animals are tested. However, if three animals died, the limit test was terminated and the main test was performed. The LD$_{50}$ is greater than 2000 mg kg$^{-1}$ if three or more animals survived. If an animals unexpectedly dies late in the study and there are other survivors, it is appropriate to stop dosing and observing all animals to see if other animals will also die during a similar observation period. The same method was adopted at limit dose of 5000 mg kg$^{-1}$.

**Hepatoprotective activity:** Rats were divided into 6 groups of 6 animals each as follows: Rats of group I served as control and received oral administration of 2.5% gum acacia (vehicle) at the dose of 1 ml kg$^{-1}$ b.wt., group II received oral administration of vehicle plus CCl$_4$ (hepatotoxic rats), group III received standard drug silymarin at the dose of 100 mg kg$^{-1}$ body weight plus CCl$_4$ as group II animals and group IV to VI received the methanolic fraction at the dose of 150, 300 and 500 mg kg$^{-1}$ body weight plus CCl$_4$ as group II animals. All treatments were given once daily for seven days.

On the 7th day, all group animals except group I, were given a single dose of CCl$_4$(1 mL kg$^{-1}$ b.wt) in 1:2 Olive oil after 6 h of last dose administration. Animals of group I were given plain doubled distilled water and olive oil in 1:2 ratio of 1 mL kg$^{-1}$ b.wt. Animals were sacrificed 24 h after the last dose and blood was collected by carotid bleeding (Kaur et al., 2006).

**Biochemical estimation:** Blood was centrifuged; serum was separated and used for estimation of biochemical parameters. Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) were estimated by Theloford, serum alkaline phosphatase (SALP) by Klin, bilirubin (total and direct) by Winsten and Cehelyk (1969) method, total protein by colour complexation with copper ion in an alkali solution (Gomall et al., 1949). All the determinations were carried out using standard kits (Agappe Diagnostic Pvt. Ltd, Kerala, India) by using fully automated Biochemical Analyser ChemWell, Awareness Technologies Inc, USA.

**Statistical analysis:** All values are expressed as Mean±SEM. Statistical analysis was performed by one-way Analysis of Variance (ANOVA) and individual comparisons of the group mean values were done using Tukey's Multiple Comparison Test, with the help of Graph Pad prism 4.0 software. The value of $p$ lower than 0.05 were considered significant.

**RESULTS**

The % yield of methanolic extract and water extract were found to be 13.82 and 1.73%, respectively. Percentage yield of fractions were found to 8.9, 7.8 and 83.3%, respectively for PER, ChF and MF.

Preliminary phytochemical investigation has revealed the presence of flavonoids, phenolic compounds, Cardiac glycosides, lactones, reducing sugars and terpenoids in the methanolic fraction. PEF found to contain sterols and terpenoids, ChF found to contain sterols, terpenoids and flavonoids and WE found to contain flavonoids, saponins, carbohydrates and Cardiac glycosides.

Total phenolic content and total flavonoid content was determined to know the ratio of phenolics and flavonoids in different fractions and extracts. Phenolic content determination is based on the on the principle
that polyphenols when react with FOLIN reagent give blue colour chromogen in alkaline media, which can be measured at 760 nm. The concentration of polyphenols in extracts is calculated by using standard curve prepared with gallic acid. The total phenolic content of methanol fraction of JG has been found to be 11.26±0.04 mg equivalent to gallic acid per 1 g of extract (sample). The Gallic acid equivalent value for PEF, CHF and WE were found to be 8.33±3.33, 9.99±1.66 and 27.77±3.46 respectively (Table 1). The principal of aluminium chloride calorimetric method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonoids. In addition, aluminium chloride forms acid labile complexes with the orthodi hydroxy groups in the A- or B-ring of flavonoids (Chang et al., 2002). The total flavonoid content of JG stem extracts was determined and reported in Table 1. The total flavonoid content of MF is more when compare to the other fractions. The value of MF was found to be 65.50±2.29 g equivalent to 1 mg of quercetin. Where as the values were found to be 17.23±0.83, 18.55±0.84 and 22.83±2.25 g equivalent to the 1 mg of quercetin respectively for PEF, CHF and WE.

### Table 1: Total phenolic and flavonoids content of the JG stem extracts (Mean±SD)

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total phenolic content (mg GAE g⁻¹ of extracts)</th>
<th>Total flavonoids (mg g⁻¹ quercetin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MF</td>
<td>112.66±0.04</td>
<td>65.50±2.29</td>
</tr>
<tr>
<td>CHF</td>
<td>8.99±1.66</td>
<td>17.23±0.83</td>
</tr>
<tr>
<td>PEF</td>
<td>8.33±3.33</td>
<td>18.55±0.84</td>
</tr>
<tr>
<td>WE</td>
<td>27.77±3.46</td>
<td>22.83±2.25</td>
</tr>
</tbody>
</table>

Free radical scavenging activity by DPPH method is shown in Table 2. MF moderately scavenged DPPH radical with the IC₅₀ value of 262.91±4.38 μg mL⁻¹. The scavenging was found to be dose dependent. Where as ascorbic acid, gallic acid and butylated hydroxyl toluene (BHT) used as standards shown IC₅₀ values of 4.17±0.5, 1.86±0.0 and 29.08±0.52 μg mL⁻¹ respectively. Where as the DPPH radical scavenging activity of PEF, CHF and WE were found to be very less and their IC₅₀ values were found to be 481.67±5.204, 482.5±5.0 and 466.67±3.145 μg mL⁻¹ respectively.

Extracts react with Fe²⁺ to reduce it to Fe³⁺. The degree of coloration indicates the reduction potential of the extracts. The change in the absorbance produced at 510 nm has been used as a measure of reducing activity of ferric ions. Reduction of all the ferric ions is considered as 100% in presence of sodium dithionite instead of the extract. Fe³⁺ reacts rapidly with 1, 10-phenanthroline and forms red (orange) coloured complex which is exceptionally stable. This complex has a strong absorption in the visible spectrum at a wavelength of 510 nm (Rajakumar and Rao, 1993). The reduction of ferric ion in presence of EDTA also determined by the method of Murthy and Mukherji (2007). As shown in Table 3, in absence of EDTA, MF has shown ferric ion reduction of 43.33±0.57% at 1000 μg concentration, where as PEF, CHF and WE shows less % of reduction than the MF. i.e., 22.71±0.66, 20.57±0.22, 40.07±0.60 respectively at the same concentration. IC₅₀ values of all fractions were not detected, where as IC₅₀ value of standards ascorbic

### Table 2: Percentage of free radical scavenging activity of JG stem extracts, ascorbic acid, gallic acid and BHT in DPPH Method (Mean±SD)

<table>
<thead>
<tr>
<th>Concentration (μg mL⁻¹)</th>
<th>MF</th>
<th>CHF</th>
<th>WE</th>
<th>Ascorbic acid</th>
<th>Gallic acid</th>
<th>BHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>05.76±0.390</td>
<td>...</td>
<td>...</td>
<td>0.25±...</td>
<td>11.12±0.315</td>
<td>16.85±1.37</td>
</tr>
<tr>
<td>100</td>
<td>12.17±1.176</td>
<td>...</td>
<td>...</td>
<td>0.50±...</td>
<td>16.95±0.57</td>
<td>22.36±1.08</td>
</tr>
<tr>
<td>150</td>
<td>17.17±1.410</td>
<td>...</td>
<td>...</td>
<td>0.75±...</td>
<td>21.91±1.62</td>
<td>28.28±2.05</td>
</tr>
<tr>
<td>200</td>
<td>34.46±1.848</td>
<td>26.13±1.117</td>
<td>20.44±0.942</td>
<td>1.00±...</td>
<td>32.81±0.97</td>
<td>36.79±1.19</td>
</tr>
<tr>
<td>250</td>
<td>47.67±1.789</td>
<td>...</td>
<td>...</td>
<td>1.50±...</td>
<td>45.20±1.61</td>
<td>42.50±1.99</td>
</tr>
<tr>
<td>300</td>
<td>58.86±1.672</td>
<td>35.52±0.981</td>
<td>25.58±0.924</td>
<td>31.75±1.057</td>
<td>2.00±...</td>
<td>58.04±0.70</td>
</tr>
<tr>
<td>400</td>
<td>43.69±0.819</td>
<td>35.06±0.709</td>
<td>42.14±1.289</td>
<td>2.50±...</td>
<td>31.70±1.52</td>
<td>...</td>
</tr>
<tr>
<td>500</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>3.00±...</td>
<td>40.02±1.85</td>
<td>...</td>
</tr>
<tr>
<td>600</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>3.50±...</td>
<td>44.30±0.45</td>
<td>...</td>
</tr>
<tr>
<td>700</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>4.00±...</td>
<td>48.20±1.03</td>
<td>...</td>
</tr>
<tr>
<td>800</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>4.50±...</td>
<td>53.99±0.37</td>
<td>...</td>
</tr>
<tr>
<td>1000</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>5.00±...</td>
<td>64.95±2.70</td>
<td>...</td>
</tr>
<tr>
<td>IC₅₀ Value</td>
<td>262.91±4.389</td>
<td>481.66±5.204</td>
<td>482.5±5.0</td>
<td>466.66±3.145</td>
<td>4.17±0.50</td>
<td>1.86±0.06</td>
</tr>
</tbody>
</table>

### Table 3: Ferric ion reduction activity of JG stem extracts, ascorbic acid and gallic acid in percentage (Mean±SD)

<table>
<thead>
<tr>
<th>Concentration (μg)</th>
<th>MF</th>
<th>CHF</th>
<th>WE</th>
<th>Ascorbic acid</th>
<th>Gallic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>14.18±0.52</td>
<td>7.11±0.22</td>
<td>5.72±0.15</td>
<td>10.99±0.52</td>
<td>2</td>
</tr>
<tr>
<td>400</td>
<td>20.91±0.66</td>
<td>10.58±0.39</td>
<td>9.62±0.34</td>
<td>17.12±0.47</td>
<td>20</td>
</tr>
<tr>
<td>600</td>
<td>29.00±0.77</td>
<td>15.60±0.55</td>
<td>12.55±0.49</td>
<td>25.74±0.86</td>
<td>30</td>
</tr>
<tr>
<td>800</td>
<td>36.33±0.97</td>
<td>18.88±0.56</td>
<td>15.91±0.58</td>
<td>32.86±1.11</td>
<td>40</td>
</tr>
<tr>
<td>1000</td>
<td>43.33±1.57</td>
<td>22.71±0.66</td>
<td>20.57±0.22</td>
<td>40.07±0.60</td>
<td>50</td>
</tr>
<tr>
<td>IC₅₀ Value</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>27.57±0.32</td>
<td>6.83±0.42</td>
</tr>
</tbody>
</table>

Concentration is the total extract/standard present in the reaction mixture in μg. ND: Not detected at the tested level, No activity was detected for the extracts/standards in presence of EDTA.
acid and gallic acid were found to be 27.57±0.32 and 6.93±0.42 μg, respectively.

H₂O₂ in the phosphate buffer has λₑₑ of 230 nm. In control tubes the absorbance will be only due H₂O₂. In presence of extracts the reduction of absorbance at 230 nm indicates scavenging or breakdown of H₂O₂. When H₂O₂ is scavenged or breakdown occurs the absorbance λₑₑ will be changed from 230 nm. As shown in Table 4, MF has scavenged the hydrogen peroxide to the extent of 30.71±4.14% at 200 μg mL⁻¹ concentration, where as PEF, ChF and WE have shown 10.64±1.59, 8.12±0.43 and 23.43±0.61 % at the same concentration. The PEF and ChF were not shown any activity up to 100 μg mL⁻¹ concentration.

In acute toxicity studies the methanolic fraction of JG extract has not shown any mortality at the limit dose of 2000 and 5000 mg kg⁻¹ b.wt. MF was found to be safe even at the higher concentration, based on this, the dose for the hepatoprotective activity were chosen.

The results obtained in the in vitro hepatoprotective activity are presented in Table 5. The hepatoprotective effect of methanolic fraction of JG was assessed using in vitro model of CCl₄ induced hepatotoxicity by the acute oxidative stress. CCl₄ affects acute oxidative injury to the liver causing oxidative damage and other changes around the central vein in the liver that leads to the leakage of the marker enzymes such as AST and ALT in the serum (Recknagel, 1997).

The activities of SGOT and SGPT after 24 h of oral administration of CCl₄ at the dose of 1 mL kg⁻¹ b.wt. are depicted in Table 5. It is observed that CCl₄ administration resulted in a significant elevation in the level of both SGOT (427.76% of the vehicle treated control) and SGPT (676.09% of the vehicle treated control). Administration of JG methanolic fraction at the 150 and 300 mg kg⁻¹ b.wt. for 7 days prior to CCl₄ administration dose dependently restored the activities of AST and ALT (Fig. 1, 2). The maximum reduction in the elevated biomarker enzymes (AST/ALT) were found to be at dose level II (300 mg kg⁻¹) and percentage of reduction was 53.29 and 25.11%, respectively. The methanolic fraction at dose level of 500 mg kg⁻¹ b.wt. has shown less hepatoprotective activity than the lower doses. These results indicate JG stem possesses moderate (maximum) antihepatotoxic activities at dose level II.

The total and direct bilirubin content present in the serum after 24 h of CCl₄ exposure is given in the Table 5. As shown in the Fig. 3 and 4 the MF at the dose of 300 mg kg⁻¹ has shown maximum activity in reducing the amount of bilirubin in the serum. The percentage of reduction was found to be 70.10 and 19.51%, respectively for total and direct bilirubin, where as the value for the silymarin were found to be 71.64 and 56.09%, respectively.

**Table 4:** Percentage of hydrogen peroxide scavenging activity of JG stem extracts (Mean±SD)

<table>
<thead>
<tr>
<th>Conc. (μg mL⁻¹)</th>
<th>MF</th>
<th>PEF</th>
<th>ChF</th>
<th>WE</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>6.04±0.61</td>
<td>ND</td>
<td>ND</td>
<td>3.12±0.53</td>
</tr>
<tr>
<td>100</td>
<td>18.47±3.20</td>
<td>ND</td>
<td>ND</td>
<td>8.74±0.97</td>
</tr>
<tr>
<td>250</td>
<td>21.40±1.45</td>
<td>3.43±0.49</td>
<td>2.18±0.51</td>
<td>15.30±1.32</td>
</tr>
<tr>
<td>500</td>
<td>30.71±4.14</td>
<td>10.64±1.59</td>
<td>8.12±0.43</td>
<td>23.43±0.61</td>
</tr>
</tbody>
</table>

**Table 5:** The hepatoprotective activity of methanolic fraction of JG stem on CCl₄ induced hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT (IU L⁻¹)</th>
<th>SGPT (IU L⁻¹)</th>
<th>Total bilirubin (mg DL⁻¹)</th>
<th>Direct bilirubin (mg DL⁻¹)</th>
<th>ALP (IU L⁻¹)</th>
<th>Total protein (gm DL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>266.50±17.36</td>
<td>119.83±4.43</td>
<td>0.41±0.06</td>
<td>0.22±0.02</td>
<td>463.83±21.36</td>
<td>6.55±0.31</td>
</tr>
<tr>
<td>CCl₄ (1 mL kg⁻¹)</td>
<td>1422.33±19.9</td>
<td>930.33±35.70</td>
<td>0.14±0.08</td>
<td>0.41±0.30</td>
<td>858.16±21.75</td>
<td>5.06±0.21</td>
</tr>
<tr>
<td>Silymarin (100 mg kg⁻¹)</td>
<td>574.33±26.50</td>
<td>440.66±26.36</td>
<td>0.55±0.04</td>
<td>0.18±0.03</td>
<td>546.83±22.81</td>
<td>6.08±0.38**</td>
</tr>
<tr>
<td>MF (150 mg kg⁻¹)</td>
<td>802.66±28.68</td>
<td>834.66±29.53</td>
<td>0.73±0.05</td>
<td>0.41±0.03</td>
<td>853±38.78**</td>
<td>5.76±0.04</td>
</tr>
<tr>
<td>MF (300 mg kg⁻¹)</td>
<td>664.33±18.92</td>
<td>696.66±15.57</td>
<td>0.38±0.03</td>
<td>0.33±0.02**</td>
<td>589±24.25*</td>
<td>6.03±0.02**</td>
</tr>
<tr>
<td>MF (500 mg kg⁻¹)</td>
<td>1202.35±37.87</td>
<td>856.66±19.22</td>
<td>0.75±0.05</td>
<td>0.41±0.03</td>
<td>797±16±48.06</td>
<td>5.53±0.16</td>
</tr>
</tbody>
</table>

Values are Mean±SEM n = 6 animals in each group. *Significantly higher significant reduction compared to hepatotoxin (CCl₄) (p<0.001), **Significant reduction compared to hepatotoxin (CCl₄) (p<0.05)
The Table 5 shows that decrease in the total protein content after CCl4 administration to about 24.81% as compared to the vehicle treated animal group in the hepatotoxic animals. The MF has restored the protein content by maximum of 20.6% at dose level II (Fig. 5).

The Serum Alkaline Phosphatase (SALP) is the one more parameter used to assess the hepatoprotective activity of MF. As given in the Table 5 the SALP has increased significantly in the CCl4 treated group when compared to the vehicle treated group. The MF has
As shown in the Table 2, MF moderately scavenged DPPH radical with the IC₅₀ value of 262.91±4.38 μg mL⁻¹. Where as ascorbic acid, gallic acid and Butylated Hydroxyl Toluene (BHT) used as standards shown IC₅₀ values of 4.17±0.5, 1.86±0.0 and 29.08±0.52 μg mL⁻¹, respectively. Ascorbic acid is a potent free radical scavenger and BHT is known antioxidant and is used as preservative (Singh et al., 2002; Mathew and Abraham, 2006). So, when compare to such potent pure compounds, IC₅₀ value of 262.91±4.38 of MF of JG is moderately high and shows that JG is a potent DPPH free radical scavenger. The results obtained are found in accordance with total phenolic content and total flavonoid content. i.e., fraction (MF) showing highest phenolic/flavonoid content showed highest free radical scavenging activity and fraction (PEF) showing lowest phenolic content showed lowest free radical scavenging activity. But though the TPC (total phenolic content) of WE was found to be more than ChF and PEF, its free radical scavenging activity found to be less than them. This may be due to the presence of phenolic or flavonoid glycosides which may not be potent radical scavengers (Amic et al., 2003; Harbone, 1984). Similarly, in ferric ion reduction capacity of different fractions the results obtained are found in accordance with total phenolic content. i.e., fraction (MF) showing highest phenolic/flavonoid content showed highest ferric ion reduction and fraction (PEF) showing lowest phenolic content showed lowest ferric ion reduction activity as in case of free radical scavenging activity. This again proves that ferric ion reduction activity of different fractions is due to the presence of phenolics and flavonoids. But in the presence of EDTA neither the extract nor the standards were shown ferric ion reduction activity. This result indicates the JG fractions and extracts reduce of ferric ions moderately, when compared to the standard ascorbic acid and gallic acid. Even in hydrogen peroxide scavenging activity, though the hydrogen peroxide scavenging activity of different fractions are less, the activity is similar to Ferric ion reduction and DPPH radical scavenging activity. i.e. as total phenolic content/flavonoid was increased hydrogen peroxide activity was found increased. These in vitro results showed that MF is a potent free radical scavenger and antioxidant among the other fractions and this activity is due to flavonoids and phenolics. Since, MF was found to be potent antioxidant it was selected for in vivo hepatoprotective activity.

The results obtained from the present study indicate that the MF exhibited significant (p<0.001) hepatoprotective effect against CCl₄ induced liver damage especially at 300 mg kg⁻¹ b.wt. by normalizing the elevated levels of the hepatic enzymes.
The ability of a hepatoprotective drug to reduce the
injurious effects or to preserve the normal hepatic
physiological mechanisms, which have been disturbed by
a hepatotoxin, is the index of its protective effect.
Protection of hepatic damage caused by
carbon tetrachloride administration has been widely used
as an indicator of liver protective activity of drugs in
general (Clausen, 1989). CCl₄ mediated hepatotoxicity was
chosen as the experimental model. It has been established
that, CCl₄ is accumulated in hepatic parenchyma cells and
metabolically activated by cytochrome P₄₅₀ dependent
monoxygenases to form a trichloromethyl radical (CCl₃). The
CCl₄ radical alkylates cellular proteins and other
macromolecules with a simultaneous attack on
polyunsaturated fatty acids, in the presence of oxygen, to
produce lipid peroxides, leading to liver damage
(Bishayee et al., 1995). Thus antioxidant or free radical
generation inhibition is important in protection against
CCl₄ induced liver lesion (Castro et al., 1974). Serum
Glutamic Oxaloacetic Transferase, Serum Glutamic Pyruvic
Transaminase, alkaline phosphatase, total and direct
Bilirubin in plasma have been reported to be sensitive
indicator of liver injury (Molander et al., 1955). The
disturbance in the transport function of the hepatocytes
as a result of hepatic injury causes the leakage of enzymes
from cells due to altered permeability of membrane. This
results in decreased levels of AST, ALT, Bilirubin and
alkaline phosphatase in the hepatic cells and a raised level
in serum. The present study revealed a significant
increase in the level of SGOT, SGPT, SALP and serum
bilirubin after exposure to the CCl₄, indicating
considerable hepatocellular injury.

It has been hypothesized that one of the principal
causes of CCl₄ induced liver injury is lipid peroxidation
induced by free radical derivatives of CCl₄. Thus,
antioxidant activity or the inhibition of the generation of free
radicals is important in the protection against CCl₄
induced liver injury (Castro et al., 1974). As MF was
found to be potent antioxidant its hepatoprotective
activity was found to be due its antioxidant and free
radical scavenging activity. All these studies show that,
the MF of Justicia gendarussa has a significant
hepatoprotective activity, which can be correlated to its
antioxidant and free radical scavenging activity.

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REFERENCES

Structure-radical scavenging activity relationships of
1st Edn., Publication and information Directorate,
CSIR, New Delhi, pp. 312.
Bishayee, A.A., Sarkar and M. Chatterjee, 1995. The
hepatoprotective activity of carrot (Daucus carota
L.) against carbon tetrachloride intoxication in mouse
Castro, J.A., G.C. De-Ferry, C.R. De-Castro,
Prevention of CCl₄ necrosis by inhibitors of drug
metabolism. Further studies on metabolism of their
Simple aromatic amines from Justicia gendarussa
¹³C NMR spectra of the bases and their analogues.
Tetrahedron, 38: 1797-1802.
Estimation of total flavonoid content in propolis by
two complementary calorimetric methods. J. Food
et al., 2002. Antioxidant activity and free radical
scavenging capacity between Korean medicinal
plants and flavonoids by assay-guided comparison.
of Indian Medicinal Plants. 1st Edn., CSIR, New
Delhi, pp: 146.
tetrachloride hepatotoxicity. Pathol. Immunopathol.
Res., 8: 104-112.
Devprakash, 2000. Lignans and Other Chemical
Components of Justicia gendarussa and Justicia
simplex and Their Bioactivity Studies. MAHE
Deemed University. Manipal.
15 Edn., Division of Reed Elsevier India Pvt. Ltd.,
Firar, I.L., 2006. Organic Chemistry-Stereochemistry and
the Chemistry of Natural Products. 5th Edn., Vol. 2,
Dorling Kindersley (India) Pvt. Ltd., New Delhi, India.
Determination of serum proteins by means of the