Pharmacological Studies on Aerial Parts of Calotropis Procera


Medicinal. Aromatic and Poisonous Plants Research Center, and Department of Pharmacology, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh-11451, Saudi Arabia.

(Accepted for publication April 1, 1991)

Abstract: The decoction of the aerial part of Calotropis procera is commonly used in Saudi Arabian traditional medicine for the treatment of variety of diseases including fever, joint pain, muscular spasm and constipation. The present investigation was undertaken to confirm its claimed activity in traditional medicine. The ethanol extract of the plant was tested on laboratory animals for its antipyretic, analgesic, anti-inflammatory, antibacterial, purgative and muscle relaxant activities. The results of this study showed a significant antipyretic, analgesic and neuromuscular blocking activity. On smooth muscle of guinea pig ileum, the extract produced contractions which was blocked by atropine supporting its use in constipation. The extract failed to produce significant anti-inflammatory and antibacterial activities. Our phytochemical studies on the aerial parts of C. procera showed the presence of alkaloids, cardiac glycosides, tannins, flavonoids, sterols and/or triterpenes. However, the chemical constituents responsible for the pharmacological activities remains to be investigated. The safety evaluation studies revealed that use of extract in single high doses (up to 3g/kg) does not produce any visible toxic symptoms or mortality. However, prolong treatment (90 days) causes significantly higher mortality as compared to control group.

Calotropis procera (Aiton) W.T. Aiton (Asclepiadaceae), is a desert plant known as Ushar or Madar in Greeco-Arab medicine. This plant is widely distributed in tropical and subtropical Africa and Asia (Miller & Morris, 1987). The medicinal use of this plant was known to ancient Egyptians, the excavation at Helwan in Egypt showed that the plant was in use in Neolithic period in Egypt (Greiss, 1955). In 'Berliner' papyrus the plant is recommended for the treatment of nodular leprosy (Ebbell, 1935). The decoction of the plant is used in Indian traditional medicine for the treatment of painful muscular spasm, dysentery, fever, rheumatism, asthma and as an expectorant and purgative (Quisumbing 1978, Chopra et al, 1956 and Nadkarni 1954). A large number of scientific studies have been undertaken...
on various parts of this plant during past decade. Prakash et al. (1998) showed that *C. procera* adversely affects early and late pregnancy in rats. The extract of this plant has been reported to possess anticancer (Ayoub and Kingston 1981), antibacterial (Malik and Chughtai 1979), nematocidal (Nandal and Bhatti, 1983) and larvicidal (Girdhar et al 1984) activities. Mascolo et al (1988) observed a highly significant antimicrobial and anti-inflammatory activity of the flowers of *C. procera*.

A recent survey of different regions of the Kingdom of Saudi Arabia showed that roots and aerial parts of *C. procera* are commonly used in traditional medicine for the treatment of variety of diseases including constipation, fever, joint pain and muscular spasm (Al-Yahya et al, 1990). The present investigation was undertaken to confirm the above said folklor claims. The parameters included antipyretic, analgesic, anti-inflammatory and antimicrobial activity of the ethanol extract of aerial part of the plant. The effect of extract on isolated smooth and skeletal muscles also studied. The safety evaluation studies (acute and chronic toxicity) were also undertaken. The plant has also been analyzed for the presence of medicinally active constituents.

Methods

*Plant Material*

The Plant materials used in this study were collected at the flowering stage from central Saudi Arabia and identified by the Taxonomy Division of the Medicinal, Aromatic and Poisonous Plant Research Center of King Saud University, Riyadh. A specimen of plant has been preserved in our herbarium (voucher number 12491) for future reference.

Shade-dried powdered aerial parts were extracted for 72 h with 96% ethanol using a Soxhlet extractor. The solvent was evaporated at low temperature under reduced pressure in a rotavapor (yield 4.1%) and stored at 4°C. The dried extract was freshly dissolved in distilled water before administration. All doses are expressed in terms of extract weight.

*Pharmacological Studies*

*Antipyretic activity in mice*

Hyperpyrexia was induced in mice by 20 ml/kg s.c. administration of a 20% aqueous suspension of brewer's yeast (Loux et al., 1972). These animals were then fasted for the duration of the experiment. The rectal temperatures were taken 24 h after the yeast injection to determine the pyretic response to yeast. Temperatures taken 1 h prior to drug administration in fevered animals served as pre-drug control. Plant extract was administered orally in a dose of 500 mg/kg body weight and the temperatures were recorded at 30, 90 and 150 min following drug administration.

*Analgesic activity in mice*
The hot plate method described by Turner (1965) was used for the determination of analgesic activity. The animals were dropped gently on a hot plate maintained at 55 + 0.5°C. The reaction time was taken as the interval extending from the instant the animal reaches the hot plate till the moment the animal licks its forefeet or jumps out. The reaction time is measured to the nearest 1/5 sec. 10 min before the oral administration of the drug (500 mg/kg) and at 30, 90 and 150 min thereafter.

Anti-inflammatory activity in rats

Carragenan-induced paw edema in rats: Pedal inflammation in albino rats was produced according to the method described by Winter et al., (1962). The carrageenan injection was given (0.05 ml of 1% carrageenan sodium salt) into the right hind foot of each rat under the plantar aponeurosis. The test group of rats was treated orally with 500 mg/kg of the ethanol extract 1 h before the carrageenan injection. At the the same time, the control group was given 5 ml/kg of normal saline and the reference group was given 100 mg/kg of an aqueous solution of oxyphenylbutazone. The measurements of foot volume were done by the displacement technique using a plethysmometer (Aplex, France) immediately before and 4 h after the injection of carrageenan.

Antimicrobial Screening

The ethanol extract of the aerial parts was tested for antimicrobial activity against S. aureus, E. coli, Pr. vulgaris, Ps. aeruginosa, C. albicans and B. subtilis microorganisms (Mitscher et al., 1972). Effect on smooth muscles. The effect on smooth muscle was studied using isolated guinea pig ileum, as described by Ghosh (1984).

Effect on skeletal muscles

The effect on skeletal muscles was tested using frog's isolated rectus abdominis muscle preparation and rat phrenic nerve diaphragm preparation according to the methods described by Ghosh (1984).

Safety Evaluation Studies

Acute Toxicity

Acute toxicity test was performed on 3 groups of mice consisting of 6 animals per group. The ethanol extract was administered orally in the doses of 0.5, 1 and 3 g/kg, body weight. The behavioral changes, symptoms of toxicity and mortality were observed for 24 h (WHO, 1967).

Chronic Toxicity

A total of 40 mice (20 male and 20 female) were randomly allotted to different treated and control groups. The extract in each case was administered in drinking water in the dose of 100
mg/kg body weight per day, for a period of 3 months (WHO, 1967). The animals were observed for symptoms of toxicity and mortality. At the end of the treatment the animals were sacrificed and blood was collected by cardiac puncture for hematological analysis. The blood was analyzed for RBC, WBC, and hemoglobin level using Contraves Degicell 3100h and Haemocell 400 hematology analyzer.

Phytochemical Screening

The phytochemical screening of the aerial parts of C. procera was conducted for the determination of alkaloids, cardiac glycosides, flavonoids, tannins, coumarins, anthraquinones, saponins, volatile oil, volatile bases, cyanogenic glycosides, glucosinolates, sterols and/or triterpenes according to the methods described by Farnsworth (1966).

Results

The ethanol extract of C. procera produced significant reduction of yeast induced increase in body temperature suggesting its antipyretic activity (Table 1). There was a significant increase in reaction time of the treated mice placed on hot plate confirming analgesic activity of the extract (Table 2). Oral administration of C. procera extract to rats elicited a weak (statistically insignificant) anti-inflammatory effect on carrageenan induced paw edema (Table 3).

The extract produced contraction of the isolated smooth muscle of guinea pig ileum which was antagonized by atropine Fig 1(a). These findings suggest parasympathomimetic effect of the extract. On isolated skeletal muscle of frog's rectus abdominis and isolated phrenic nerve diaphragm preparations the extract showed neuromuscular blocking activity Fig. I (b & c). The extract failed to produce antimicrobial activity against S. aureus, E. coli, Pr. vulqaris, Ps. aeruginosa, C. albicans and B. subtilis microorganisms.

Table I. Effect of the ethanol extract of C. procera on yeast induced hyperpyrexia in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Oral Dose mg/kg body weight</th>
<th>Rectal temperature°C Predrug 30 min</th>
<th>Rectal temperature°C Postdrua 90 min</th>
<th>Rectal temperature°C Postdrua 150 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.24</td>
<td>36.43 +</td>
<td>36.41 +</td>
<td>36.33 +</td>
</tr>
<tr>
<td>(Saline)</td>
<td></td>
<td>0.22</td>
<td>0.15</td>
<td>0.06</td>
</tr>
<tr>
<td>C. procera</td>
<td>500</td>
<td>36.45 +</td>
<td>35.9* +</td>
<td>35.6* +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.15</td>
<td>0.16</td>
<td>0.14</td>
</tr>
<tr>
<td>Aspirin</td>
<td>150</td>
<td>36.23 +</td>
<td>35.53***+</td>
<td>34.55** +</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.19</td>
<td>0.26</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Six animals were used in each group.

*P < 0.05; **P < 0.01 and ***P < 0.001. Student's t-test.
Table II. Effect of the ethanol extract of C. procera on hot plate reaction time in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Oral Dose mg/kg body weight</th>
<th>Predrug Reaction time (Seconds)</th>
<th>30 min</th>
<th>90 min</th>
<th>150 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>3.85 +</td>
<td>3.66 ±</td>
<td>3.50 +</td>
</tr>
<tr>
<td></td>
<td>0.27</td>
<td></td>
<td>0.27</td>
<td>0.35</td>
<td>0.22</td>
</tr>
<tr>
<td>C. procera 500</td>
<td>3.91 +</td>
<td></td>
<td>4.00 +</td>
<td>6.66 +</td>
<td>5.85 +</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td></td>
<td>0.34</td>
<td>0.42**</td>
<td>0.43*</td>
</tr>
<tr>
<td>Aspirin 150</td>
<td>3.91 +</td>
<td></td>
<td>5.66 +</td>
<td>7.411 +</td>
<td>8.50 +</td>
</tr>
<tr>
<td></td>
<td>0.27</td>
<td></td>
<td>0.55*</td>
<td>0.37**</td>
<td>0.43***</td>
</tr>
</tbody>
</table>

Six animals were used in each group.

*P < 0.05; **P < 0.01 and ***P < 0.001. Student's t-test.

Table III: Effect of the ethanol extract of C. procera on Carrageenan induced paw edema in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose M3A9</th>
<th>Volume of paw (ml) after Carrageenan administration (mean ± S.E.)</th>
<th>+ 4 h increase in paw volume (ml)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Saline</td>
<td>1.203 ± 0.01</td>
<td>0.245 ± 0.001</td>
<td>-</td>
</tr>
<tr>
<td>C procera extract</td>
<td>500</td>
<td>1.197 ± 0.01</td>
<td>0.235 ± 0.022</td>
<td>4.09</td>
</tr>
<tr>
<td>Oxyphen-butazone</td>
<td>100</td>
<td>1.412 ± 0.017</td>
<td>0.08 ± 0.012</td>
<td>67.35</td>
</tr>
</tbody>
</table>

Six ordinals were used in each group.
**Table TV:** Effect of chronic treatment (90 days) of the ethanol extract induced hematological changes in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oral dose (mg/kg/day)</th>
<th>Haemoglobin (mg/100 ml)</th>
<th>RBC (x10⁶)</th>
<th>WBC (x10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>11.21±0.45</td>
<td>6.13±0.26</td>
<td>3.15±0.34</td>
</tr>
<tr>
<td><em>C. procera</em> extract.</td>
<td>100</td>
<td>12.47±0.31</td>
<td>6.83±0.17</td>
<td>4.12±0.57</td>
</tr>
</tbody>
</table>

Five animals were used in each group.

**Table V:** Effect of chronic treatment (90 days) of the ethanol extract of *C. procera* on the mortality in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oral dose (mg/kg/day)</th>
<th>No. of mice</th>
<th>Total of dead mice</th>
<th>Percent Lethality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>20</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td><em>C. procera</em> extract.</td>
<td>100</td>
<td>20</td>
<td>10</td>
<td>50*</td>
</tr>
</tbody>
</table>

10 male and 10 female mice were used in each group.

* P < 0.05, Student's t-test.
The acute toxicity test showed no mortality or any gross change in behavior in mice up to a dose of 3 g/kg body weight orally over a period of 24 hours. However, a fifty percent mortality of the animals was recorded upon the chronic treatment for a period of 90 days (Table 4). There was no significant change in the hemoglobin level and RBC and WBC counts of the animals (Table 5). The phytochemical study of the aerial parts of the plant revealed the presence of alkaloids, cardiac glycosides, flavonoids, tannins, saponins, sterols and/or triterpenes.

Discussion

The ethanol extract of aerial parts of C. procera produced significant analgesic, antipyretic and neuromuscular blocking activity with negligible anti-inflammatory activity. These results support the use of aerial parts for the treatment of fever, pain and muscular spasm in traditional medicine (Al-Yahya, 1990). The much claimed use of this in rheumatic disorders (Chopra et al, 1956) is not supported by our findings; however, it may relieve the rheumatic
pain by virtue of its analgesic activity.

Our findings on antipyretic and analgesic activity of this extract of aerial part of C. procera is in agreement with Mascolo et al (1988) who reported similar findings in the ethanol extract of the flowers of this plant. The results of this study suggest that the extract is different from salicylate group of drugs which shared significant anti-inflammatory activity (Higgs and Flower 1981); rather it resembles acetaminophen like-drugs which mainly possesses antipyretic and analgesic activities (Ameer and Greenblatt, 1977). The lack of anti-inflammatory activity in the later group of compounds has been attributed to their weak inhibitory activity on prostaglandin biosynthesis (Ameer and Greenblatt, 1977). Mascolo et al (1988) showed a significant release of prostaglandin following the administration of flower extract of C. procera. These authors also attributed the anti-inflammatory activity of flower extract of C. procera to its inhibitory effect on the prostaglandin synthesis.

Our findings suggest that the aerial parts of C. procera is chemically different from the flowers and possesses no action on prostaglandins. Our findings on the stimulatory effect on intestine also supports its use as purgative in traditional medicine (Kirtikar and Basu, 1935). The extract induced contractions of smooth muscle were antagonized by atropine, suggesting cholinergic action of the extract.

The result of this study showed that the C. procera extract is devoid of any antibacterial and antifungal activity. These findings clearly contradict the results of Mascolo et al (1988) who showed highly significant antimicrobial activity in the flower extract of this plant. These findings suggest that although some ingredients may be common in flower extract and total aerial parts extract but are not identical chemically and pharmacologically.

The aerial parts was found to contain various medicinally active ingredients including alkaloids, cardiac glycosides, tannins, saponins and/or triterpenes. However, the nature of the pharmacologically active principles require further studies. Our studies on acute toxicity tests suggest the plant may be used safely in single high doses. However, the prolong use of this plant may result in serious health hazards as is evident from our chronic toxicity studies.

Acknowledgement

The authors are thankful to King Abdul Aziz City for Science and Technology, Riyadh for financial assistance and Mr. Altaf H. Naqvi for typing the manuscript.

References