Evaluation of In-Vivo Anti-inflammatory activity of leaves extract of Carissa spinarum on Formalin Induced Paw Edema in Albino Rats

Neeli Rose Beck*, Kamta Prasad Namdeo

SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur-495001, Chhatisgarh, India

Abstract

The present study was undertaken to evaluate the anti-inflammatory activity of the leave extracts of Carissa spinarum. In ancient literature the medicinal values of Carissa spinarum and it is useful in treatment of rheumatism, purgative and snake repellent. Dried pulverized leaves of C.spinara were extracted with petroleum ether, chloroform, ethanol and distilled water by using soxhlet apparatus separately. Formalin induced rat hind paw edema method was used for determination of anti-inflammatory activity. Group I was taken as normal control and the group II as formalin control. Group III was treated with the standard drug analgin. The petroleum ether extract, chloroform extract, alcoholic extract and aqueous extract of C. spinarum leaves 200 mg/kg were feed to group IV, V, VI and VII respectively. All extracts exhibited significant anti-inflammatory activity, but aqueous extract produces maximum activity compared to other extracts of C. spinarum. It has been concluded that aqueous extract of C. spinarum leaves having good anti-inflammatory activity against formalin induced rat paw edema.

1 Introduction

Inflammation is generally considered to be a normal response to protect the injured tissue caused by physical trauma, and other harmful chemical or microbiological agents. The redness, swelling, pain, heat and weakness are some of the symptoms of inflammation.

Prostaglandins are substance that indicates and modulates cell and tissue responses involved in inflammation. During an inflammatory response, mediators, such as pro-inflammatory cytokines, including interleukin-1, tumour necrosis factor (TNF), interferon (INF)-c, IL-6, IL-12, IL-18 and stimulating factors are released as the granulocyte macrophage colony. And antiinflammatory cytokines prevents such responses like IL-4, IL-10, IL-13, IFN-a and transforming growth factor beta and others. Their biosynthesis also cause disorders like cardiovascular disease, cancer, alzheimer’s etc. Some of the synthetic drugs increase the effects of adverse cardiovascular thrombotic effects such as Cox-2 inhibitors and non steroidal antiinflammatory drugs. Synthetic drugs are available in high costs and causes severe adverse reactions and toxicity, including some risk of infections in patients during treatment. Hence it is emphasised in scientific exploration of herbal drugs being the less side effects to overcome them. This is very evident in the modern medical practices that medicinal plants are extensively used in treatment of various diseases. Alone in India about 75% of the population relies for their healthcare on herbs and herbal products. Carissa spinarum roots are used in rheumatism, purgative and snake repellent. Many researchers have been done pharmacological studies on Carissa spinarum. An account of their work they found their positive biological activities like antiarthritic, anticonvulsant, hepatoprotective, antioxidant, anthelmintic and wound healing. Chemical compositions of Carissa spinarum are fatty acids, viz., palmitic, stearic, oleic, arachidic and linoleic acids. It contains volatile oils, like triterpene, ursolic acid, myrcene, limonene, camphene, dipentene, farnesol, α-terpeneol, citronellal, β-ionone, carissol, linalool and geranyle acetate. It also contains carbohydrates, reducing and non reducing sugars, galactose and glucose. Other phytoconstituents are tannins, pectin, vitamin C, carissone, carindone, carinol, odoroside H, digiotoxigenin, D-digitalose are present in Carissa spinarum extract. Hence, this study was undertaken to evaluate the anti-inflammatory activity of
Carissa spinarum leaves extracts against formalin induced paw edema in albino rats.

2 Materials and Methods

2.1 Plant collection and identification

The plant specimens were collected in the month of January from the Jashpur District of Chhattisgarh. Plant specimen was identified and authenticated by Botanist from department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh, India. Voucher specimen is SLT/Med.Plant/03/2009 was deposited in the department of Pharmacognosy, Guru Ghasidas Vishwavidyalaya, Bilaspur and Chhattisgarh.

2.2 Preparation of extracts

Plant leaves were cleaned, dried under shade and powdered by a mechanical grinder. Hundred grams of ground powdered leaves were extracted with petroleum ether, chloroform, ethanol and distal water in a soxhlet apparatus successively. The extracts were filtered separately and concentrated by vacuum pressure. The yield of petroleum ether, chloroform, ethanol and aqueous extract was found to be around 1.12%, 0.61%, 5.63% and 9.88% (W/W) respectively. Extracts were suspended in 1% acacia solution for the pharmacological assay.

2.3 Phytochemical analysis

Phytoconstituents were identified by various chemical tests and found that alkaloids, tannins, fats and fatty acids, flavonoids, carbohydrates and steroids are present in leaves extracts of Carissa spinarum\(^{17}\) (Table 1).

2.4 Animals and experimental protocol

Albino rats of both sexes weighing between 200-250 gm were used for experiment. They were housed in standard environmental condition. Animals were given standard pellet and water in free access. All procedures and protocols were approved by the Institution of Animal Ethics Committee (IAEC) and registration number is 994/a/Go/06/CPCSEA. All animal experiments were carried out strictly in accordance with the guidelines of CPCSEA.

2.5 Determination of anti-inflammatory activity

In present study formalin induced rat hind paw edema method was used for determination of anti-inflammatory activity. Analgin was used as standard drug.

2.6 Formalin induced rat hind paw edema method

Albino Wister rats (200-250 gm) were used for the study. The animals were divided into seven groups of six rats in each group. Inflammation was produced in animals by injection of 0.1 ml of 1% w/v formalin into the sub plantar region of left hind paw\(^{18}\). Group I served as normal control animals administered distilled water. Group II formalin control animals administered formalin (0.1 ml). Group III standard animal administered formalin (0.1 ml) and analgin (30 mg/kg). Group IV animal administered formalin (0.1 ml) and petroleum ether extract (200 mg/kg). Group V animal administered formalin (0.1 ml) and chloroform extract (200 mg/kg). Group VI animal administered formalin (0.1 ml) and alcoholic extract (200 mg/kg). Group VII animal administered formalin (0.1 ml) and aqueous extract (200 mg/kg). The paw edema volume was measured mercury displacement technique using plethysmograph at 0, 15, 30 and 60 min after formalin injection. The difference between the initial and subsequent values gave the actual edema volume which was compared with control.

Table 01: Phytochemical analysis of Carissa spinarum leaves extracts

<table>
<thead>
<tr>
<th>Test</th>
<th>PEECSL</th>
<th>CHECSL</th>
<th>EECSL</th>
<th>AQECSL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycoside</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrate &amp; sugars</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fats &amp; fixed oil</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Protein &amp; amino acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gums and mucilage</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Positive (+), Negative (-). PEECSL: Petroleum ether extract of C.spinarum leaves, CHECSL: Chloroform extract of C.spinarum leaves, EECSL: Ethanol extract of C. spinarum leaves, AQECSL: Aqueous extract of C.spinarum leaves.
Where $D_0$ was the average inflammation of the group of rats after 15 min treatment of formalin

$D_t$ was the average inflammation of the drug treated rats after 30 and 60 min.

### 2.7 Statistical analysis

All values of experiment are expressed as Mean ± SEM. The data obtained from pharmacological screening were statistically analysed by one way analysis of variance (ANOVA) followed by Dunnett’s comparison test using Graph pad Instat. $P < 0.01$ was considered to be statistically significant.

### Table 2: Anti-inflammatory activity of various extracts of Carissa spinarum leaves

<table>
<thead>
<tr>
<th>Group and Dose</th>
<th>0min (before treatment of formalin)</th>
<th>15min after Formalin treatment of paw volume</th>
<th>30 min after drug administration of paw volume</th>
<th>60min after drug administration of paw volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (distal water)</td>
<td>0.423±0.014</td>
<td>0.423±0.004**</td>
<td>0.422±0.001**</td>
<td>0.420±0.003**</td>
</tr>
<tr>
<td>Formalin (1%) (0.1ml)</td>
<td>0.468±0.012</td>
<td>0.921±0.0025**</td>
<td>0.92±0.0021**</td>
<td>0.912±0.001**</td>
</tr>
<tr>
<td>Formalin + Standard (Analgin 30mg)</td>
<td>0.436±0.080</td>
<td>0.983±0.006**</td>
<td>0.835±0.003** (15.05%)</td>
<td>0.65±0.001**</td>
</tr>
<tr>
<td>Formalin + Petroleum ether extract of leaves (200 mg)</td>
<td>0.482±0.011</td>
<td>0.945±0.002**</td>
<td>0.934±0.001** (1.16%)</td>
<td>0.90±0.003** (4.76%)</td>
</tr>
<tr>
<td>Formalin + Chloroform extract of leaves (200 mg)</td>
<td>0.456±0.002</td>
<td>0.842±0.0012**</td>
<td>0.805±0.0012** (4.39%)</td>
<td>0.786±0.002** (6.65%)</td>
</tr>
<tr>
<td>Formalin + Alcoholic extract of leaves (200 mg)</td>
<td>0.458±0.016</td>
<td>0.894±0.001**</td>
<td>0.852±0.008** (4.69%)</td>
<td>0.832±0.008** (6.93%)</td>
</tr>
<tr>
<td>Formalin + Aqueous extract of leaves (200 mg)</td>
<td>0.462±0.008</td>
<td>0.921±0.0008**</td>
<td>0.895±0.40** (2.82%)</td>
<td>0.764±0.004** (17.04%)</td>
</tr>
</tbody>
</table>

Result expressed as Mean ± SEM from six observations ** indicate $P<0.01$ significant.

### 4 Discussions

Inflammation is a natural complex protective biological response by the living organism to remove harmful stimuli such as pathogens, damaged cells or irritant and to initiate the healing process for tissues. The inflammatory tissues are damaged because liberation of reactive oxygen substances from phagocytes in the inflammation sites. Formalin induces paw oedema is closely resembles human arthritis. The inflammatory effect of formalin is biphasic; neurogenic component response and tissue mediated response. An early neurogenic response is mediated by substance P and bradykinin followed by a later tissue mediated response where histamine, 5HT, prostaglandins, cyclooxygenase and bradykinin serotonin are involved. Some other chemicals interleukin, interferon, tumour necrosis factors granulocytes macrophage colony – stimulating factors are released. Liberation of these chemical mediators is responsible for pain and inflammation. The antiinflammatory property of plant extracts could be due to the neutralization of chemical mediators like histamine, serotonin etc.

In this present study result exhibits the paw volume is decreased when time is increased. It means that reduction of the swelling of inflammatory sites by inhibiting release of chemical mediators like histamine, bradykinin, serotonin & prostaglandin etc. Literature reveals that the phytoconstituents like terpenoids and steroidal saponins, tannins and flavonoids which had been reported to possess anti-inflammatory property. These phytoconstituents possess antiinflammatory effects through inhibiting prostaglandin pathways. Flavonoids are act as phospholipase inhibitors and...
some have been reported as TNF-α inhibitors in different inflammatory condition. It is also reported that flavonoids have properties depending upon their chemical nature it can inhibit both cyclooxygenase and lipoxygenase pathways of arachidonic metabolism. All test groups was shown positive antiinflammatory activity. Phytoconstituents tannins and flavonoids are present in aqueous, alcoholic and chloroform extracts of Carissa spinarum leaves. Maximum activity was observed in aqueous extract (17.04% inhibition) of Carissa spinarum may be due to presence of high concentration of phytoconstituents tannins and flavonoids are found. Antinflammatory activity of this plant may be due to tannins and flavonoids which are inhibited pathways of prostaglandin and arachidonic metabolism. The result obtained from the experiment it is concluded that the Carissa spinarum have potent anti-inflammatory property.

**Fig 1: Anti-inflammatory activity of Carissa spinarum leave extracts**

Results supports scientifically after experiment that the plant used for treatment of swelling traditionally. Phytoconstituents which are present in plant may be helpful for further investigation in pharmacological activities, its mechanism of action and elucidation of various phyto molecules in near future.

5 Competing interests

This research received no specific grant from any funding agency. The authors declare no conflict of interest.

6 Author’s contributions

NRB carried out literature review, data collection and manuscript preparation. KPN participated in collection of data and manuscript editing. Both authors read and approved the final manuscript.

7 References


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