ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF MORINDA CITRIFOLIA ROOT EXTRACTS

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ABSTRACT

The study was designed to evaluate the anti-inflammatory and analgesic activities of ethanolic extract of roots of Morinda citrifolia Linn. (Rubiaceae). The anti-inflammatory potential of ethanolic extract has been determined by using carrageenan-induced paw edema assay in albino rats. The analgesic activity was tested by using acetic acid-induced writhing response and hot plate method in albino mice. The administration of extract at doses of 100 and 200 mg/kg, by oral administration, significantly inhibited carrageenan-induced inflammation. Also, the acute treatment of extract produced a significant antinociceptive effect in the acetic acid-induced writhing, and hot-plate-induced pain. The experimental data demonstrated that ethanolic extract of roots of Morinda citrifolia Linn. possess remarkable anti-inflammatory and analgesic activities.

KEYWORDS: Morinda citrifolia, antinociceptive, Rubiaceae.

INTRODUCTION

The use of medicinal plants and herbs has recently been increased throughout the world for the maintenance and improvement of health and for the treatment of various human conditions and diseases. Noni (Morinda citrifolia L.), belonging to the family Rubiaceae, is an indigenous tree of the tropical zones of South Asia, Australia, Hawaii, and the islands of French Polynesia. Its fruit, leaves, seeds, bark, and roots have been traditionally used for prevention or improvement of various diseases, including arthritis, infections, colds, cancers, diabetes, etc. [1] Traditionally, the roots of Noni plants were used by Polynesians to produce yellow or red dye, but more importantly, they are now known to contain medicinally active components, such as anthraquinones, which, due to its antioxidant activities, possess various
therapeutic properties. These include anti-bacterial, anti-viral, cytotoxic and immunomodulatory activities and anti-cancer activities as well as analgesic effects. This makes the compounds potentially useful in several medical applications[3,4,5] while the leaves used for the treatment of wound infections, arthritis, swellings, and similar conditions[6,7] The major active phytoconstituent present in the in the Noni plant are scopoletin, octoanoic acid, potassium, vitamin C, terpenoids, alkaloids, anthraquinones (such as nordammacanthal, morindone, rubiadin, and rubiadin-1-methyl ether, anthraquinone glycoside), b-sitosterol, carotene, vitamin A, flavone glycosides, linoleic acid, Alizarin, amino acids, acubin, L-asperuloside, caproic acid, caprylic acid, ursolic acid, rutin, and proserine.[8,9,10]

Pain is a pathophysiological response of living tissue to undesirable stimuli evoked by external or internal noxious stimulus. Pain is warning signal though protective in nature, but causes discomfort and suffering. The pharmacology of pain is a complex phenomenon.[11,12] Inflammation is the local response to living tissue to injury due to any agent. The agent causing inflammation may be as under: a) Physical agent like heat, cold, radiation, mechanical trauma. b) Chemical agents like organic and inorganic poisons. c) Infective agents and their toxins like from bacteria and viruses. d) Immunological agents like cell-mediated and antigen-antibody reaction.[13] The analgesic activity of noni fruit puree on mice was investigated by Basar et al. (2010) using the hot plate test. A 10% solution of freeze concentrated noni fruit puree in the drinking water of mice reduced the pain sensitivity comparably to the central analgesic drug tramadol. The findings suggest that the preparations of noni fruits are effective in decreasing pain and joint destruction caused by arthritis.[14]

French research team by Younos, valuated the analgesic and sedative effects of lyophilised aqueous extracts of roots of Morinda citrifolia plant. The extract showed significant, dose-related, central analgesic activity in the extract treated mice when compared to morphine (as sulfate 1.15mg/kg i.p) The analgesic efficacy of the Noni extract is 75% as strong as morphine, yet non-addictive and side effect free.[15]

More recently, completely synthetic compounds based on natural pharmacophores have been introduced into the market but, research and medical fields still struggle with side-effect profiles from these analgesic substances that are undesirable. Therefore, development of newer and more substantial analgesic drugs with lesser side-effects is necessary. Alternative medicine are of less toxicity and with fewer side effects compared with conventional medicine, and hence it is important to introduce a scientific validation for the medicinal effect
of plants used in traditional medicine. In the previous study, *Morinda* citrifolia fruit and leaf were reported to possess a significant analgesic effect on animal models. However, the literature survey revealed that no systematic study had been carried out on root. Hence, the present study was planned to evaluate the possible analgesic and anti-inflammatory potential of ethanolic root extract of *Morinda citrifolia* using different experimental models through bioassay-guided procedures.

**MATERIALS AND METHODS**

**Collection and Treatment of Plant Material**

The root part of *Morinda citrifolia* plant was collected from Quereshi bagh nursery, Jamnagar, Gujrat and was authenticated by agricultural university, Nagpur, Maharashtra. Authentication No. 9496.

**Extraction of Morinda citrifolia Root**

The fresh roots of M. citrifolia were harvested, washed and dried under shade, broken into small pieces and powdered coarsely. 1000gm of powdered root was extracted in soxhlet apparatus with ethanol (99.9% v/v) for 72 hrs. The extract was concentrated to dryness under reduced pressure and controlled temperature (40-50° C) using rotary evaporator[16] The ethanolic extract yielded an brown sticky mass weighing 32g and yield was found to be 3.2% w/w.

**Preliminary Phytochemical Analysis**

The freshly prepared extract (MCREx) was qualitatively phytochemically tested for the presence of various phytoconstituents including steroids, flavonoids, tannins, phenols, glycosides, flavonoids, carbohydrate and anthraquinones using standard protocols.[17] MCREx tested positive for the presence of flavonoids, amino acids, carbohydrate, tannins, phenols, alkaloids and saponins were also found present.

**Acute oral toxicity studies**

Acute oral toxicity assay was performed in healthy nonpregnant adult female albino Swiss mice (20–35 g) and albino Wistar rats (200–250) divided into different groups as per the OECD guidelines-423 (OECD, 2001). Two groups of three albino Swiss mice and albino Wistar rats were treated with MCREx 2000 mg/kg orally. The control group received 2% CMC suspension at the same volume. In the acute oral toxicity test dose of 2000 mg/kg of MCREx did not cause mortality in mice and rats during 14-days observation. The mice and
rats did not show any signs of toxicity or change in general behavior or other physiological activities.

**Experimental animal**

Male and female albino mice (25–30 g) and Wistar albino rats (200–250 g) were used. Animals were housed under standard conditions (i.e. at 22 ± 2 °C, humidity: 50–55% and 12 h natural light/dark cycle) and fed with standard pellet diet and water ad libitum. Each of these treatment groups consisted six animals/group. The protocol of the study was approved by Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), approval no. (CPCSEA/IAEC/PC-02/06-2K9).

**Analgesic activity**

**Hot plate reaction time in mice**

Mice were screened by placing them on a hot plate (Columbus, USA) maintained at 55 ± 1.0 °C and the reaction time in seconds for hind paw licking or jumping were recorded. Only mice which reacted after 4-6 seconds and which did not show large variation were used in this study. Each mouse served as its own control. The mice were orally administered the test drug (MCREx 100 and 200 mg/kg). Morphine (5 mg/kg s.c.) was used as standard. Before treatment, the time for hind paw licking or jumping on the heated plate of analgesiometer was determined thrice at 1 h intervals and mean of these taken as the initial reaction time (control latency). Mice in each group were tested 30, 60 and 90 min after drug treatment. A latency period of 30 seconds was defined as complete analgesia as cut off time to prevent damage to mice.

**Acetic acid induced writhing method**

Albino mice of either sex were divided into 4 groups of 6 animals each. Extracts were orally administered. Group I served as control and received 1% tween 80 at the dose of 1ml/kg. Group II served as standard and received Morphine (5 mg/kg s.c.). Group III and Group IV received test drug (MCREx 100 and 200 mg/kg) respectively. After 30 minutes of drug administration acetic acid (1% v/v) was administered to all group of animals at a dose of 1 ml/100g intraperitoneally. The onset and severity of writhing response was noted for 10 minutes. The inhibition of pain response by drug treatment was noted.\(^{19}\)

**Anti-inflammatory activity**
Rats were divided into 4 groups (6 animals in each group). Animals of all the groups were injected with 0.1 ml of 1% carrageenan in normal saline, under the plantar region of the right hind paw. Group I animals (carrageenan control) received suspension of 1% of Tween 80 p.o., 30 min prior to carrageenan injection. Group II, the standard reference group was given p.o., an aqueous solution of indomethacin (5 mg/kg), 30 min prior to carrageenan injection. Group III and Group IV received p.o., 100 and 200 mg/kg of MCREx extract suspension in 1% of Tween 80, 30 min prior to carrageenan injection, respectively. The paw volume of the rats was measured plethysmographically just before and 3 h after carrageenan injection. The anti-inflammatory activity was determined as the percentage of inhibition of inflammation after it was induced by carrageenan by taking volume of inflammation in control group as 100%. The percentage inhibition was calculated.

**Statistical Analysis**

Results are expressed as the mean ± SD. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range Test. The value, p<0.05 is considered as statistically significant when compared with control group.

**RESULTS**

Table 1: Effect of the ethanolic extract of roots of *M.citrifolia*. (100 and 200 mg/kg) on acetic acid induced writhing in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Number of writhings (10 min)</th>
<th>Percentage Inhibition in writhing response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1% acetic acid)</td>
<td>1ml/100g</td>
<td>48.12±0.14</td>
<td>----</td>
</tr>
<tr>
<td>Morphine</td>
<td>5mg/kg</td>
<td>17.24±0.15**</td>
<td>64.17</td>
</tr>
<tr>
<td>MCREx 100mg/kg</td>
<td>24.02±0.16*</td>
<td>50.08</td>
<td></td>
</tr>
<tr>
<td>MCREx 200mg/kg</td>
<td>19.31±0.31*</td>
<td>59.87</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01 vs. control. Values are mean ± SE from 6 animals in each group.

Table 2: Effect of ethanolic extract of roots of *M citrifolia*. (100 and 200 mg/kg) on mice tested for the hot plate test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Reaction time before drug treatment</th>
<th>Reaction time after 15 min</th>
<th>Reaction time after 30 min</th>
<th>Reaction time after 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1ml/100g</td>
<td>6.45±0.22</td>
<td>6.78±0.42</td>
<td>7.01±0.01</td>
<td>6.35±0.43</td>
</tr>
<tr>
<td>Morphine</td>
<td>5mg/kg</td>
<td>7.00±0.32</td>
<td>13.00±0.02**</td>
<td>14.23±0.32**</td>
<td>14.51±0.15**</td>
</tr>
<tr>
<td>MCREx 100mg/kg</td>
<td>6.56±0.31</td>
<td>9.01±0.12*</td>
<td>10.22±0.12*</td>
<td>10.74±0.18*</td>
<td></td>
</tr>
<tr>
<td>MCREx 200mg/kg</td>
<td>7.11±0.21</td>
<td>9.56±0.014*</td>
<td>10.00±0.16*</td>
<td>11.58±0.28*</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01 vs. control. Values are mean ± SE from 6 animals in each group.
Table 3: Effects of ethanolic extract of roots of *M. citrifolia*. (100 mg/kg, 200 mg/kg) and Indomethacin (10 mg/kg) on carrageenan-induced rat paw edema.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oral Dose mg/kg</th>
<th>Difference in paw volume at 3 h (ml)</th>
<th>Percentage inhibition of oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.45 ± 0.01</td>
<td>---</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>5mg/kg</td>
<td>0.11 ± 0.02**</td>
<td>75.55</td>
</tr>
<tr>
<td>MCREx</td>
<td>100mg/kg</td>
<td>0.28 ± 0.01*</td>
<td>37.77</td>
</tr>
<tr>
<td>MCREx</td>
<td>200mg/kg</td>
<td>0.21 ± 0.04*</td>
<td>53.33</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01 vs. control. Values are mean ± SE from 6 animals in each group.

DISCUSSION

In the present study ethanolic extract of roots of *Morinda citrifolia* (MCREx) at the doses of 100–200 mg/kg protected mouse against both chemical and thermal-induced noxious stimuli, which were evidenced from the acetic acid-induced writhing and hot plate tests. Acetic acid induced writhing test is widely used method for evaluation of peripheral antinociceptive activity. Acetic acid is an irritating agent which stimulate local peritoneal receptor to induce pain with characteristic abdominal constriction when injected into peritoneal cavity. In present study, MCREx markedly reduced number of mouse abdominal constriction. The extract showed dose dependent inhibition of acetic acid induced writhing in mice (Table 1). Hot plate test was also assayed to characterize the analgesic activity of extract. It is possible that ethanol extract exerts analgesic effect probably by inhibiting the synthesis of prostaglandins. Animal produced paw licking and paw jumping in the control group, and MCREx at both doses (100 and 200 mg/kg) used in the study significantly inhibited the jumping and licking response in mice. Morphine (5 mg/kg s.c.) dose was significantly reduced the paw licking and paw jumping response when compared to the control (Table 2). Inflammation is a complex process and various mediators e.g. prostaglandins, leukotrienes and kinins, platelet activating factor, etc. have been reported to be involved in the development if inflammatory diseases. Oral administration of MCREx significantly inhibited (Table 3) the carragenan induced paw oedema in rat at both doses (100 and 200 mg/kg).

CONCLUSION

Roots of *Morinda citrifolia* Linn. contains alkaloids, flavonoids, amino acids, carbohydrate, tannins, phenols, and saponins. Flavonoids have been shown to possess various biological properties related to antioxidant, antinociceptive, and anti-inflammatory activity by targeting reactive oxygen species and prostaglandins which are involved in the late phase of acute
inflammation and pain perception. It can be concluded from study that ethanolic extract of *M. citrifolia* possess anti-inflammatory and antinociceptive activities may be due to the presence of flavonoids and other polyphenolic moieties present in it, which seems to support the use of this plant in traditional medicine.

**REFERENCE**


hydroalcoholic extract of Areca catechu L. nut., Food and Chemical Toxicology., 2010; 48: 3412–3417.


