Analgesic Activity of Carbazole Alkaloid From \textit{Murraya paniculata} Linn. (Rutaceae)

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\textbf{Abstract:} \textit{Murraya paniculata} Linn. (Rutaceae) is commonly used in the treatment of pain, rheumatism diarrhea and dysentery. Leaves of this plant were standardized pharmacognostically. Preliminary phytochemical study of methanolic extract showed the presence Alkaloids, Flavonoids, Phenolic compounds, Phytosterols, Proteins and amino acids while saponins were absent Phytochemical studies led to isolation of alkaloid Isomurrayafoline B (Isb) and was tested at 10 and 20 mg/kg p.o. for \textit{in vivo} analgesic activity, peripheral as well as central pain model. Isb demonstrated 80.33\% and 93.87\% inhibition in the number of writhes at both doses respectively. Similarly, the thermal attenuation of Isb (20 mg/kg p.o.) was found more significant ($P<0.01$) after 60 min of administration that remained significant upto 120 min, while Isb (10mg/kg p.o) showed significant effect ($P<0.01$) only after 60 minutes of administration. The isolated compound showed profound analgesic activity in both peripheral and central pain models; needs further detail studies for clinical utility. In the meantime, the study validated the uses of the plant in traditional system of treatment.

\textbf{Key words:} \textit{Murraya paniculata} • Isomurrayafoline B (Isb) • Analgesic Activity

\textbf{INTRODUCTION}

Pain sensation is a series of complex interactions between central nervous system and peripheral nervous system. It is obviously a complex multiage health problem which needs prompt attention as altering physical, psychological and overall quality of life. Such conditions are generally characterized as a protective modality and appear as a significant tool for the diagnosis of different pathological conditions. However, management of chronic pain is considered as a great challenge for most of clinicians while the diagnosis and treatment of acute pain can be a difficult task [1, 2] and ultimately led to the concept of “\textit{Fear of Pain}”. Chronic pain becomes hard task for patients to manage due to risks of toxicity related to drugs. Drugs which are in use presently for the management of pain and inflammatory conditions normally causes gastrointestinal damage and other side effects[3]. Herbal medicines are being widely used in the world because of better cultural accept ability, least injurious with none or much reduced side effects [4].

\textit{Murraya paniculata} Linn. (Synonyms: \textit{Murraya exotica} L.) Belongs to the family Rutaceae and is commonly known as orange jasmine. \textit{M. paniculata} (L.) Jack is an evergreen shrub, grows commonly in the plain areas through the country[5]. The \textit{M. paniculata} is a native and common throughout much of India, Pakistan, China, Burma and Malacca and dry areas of Ceylon and is often grown in Thailand, Cambodia, South Vietnam and East Africa, Indonesian, Brazil and Philippines [6]. In traditional system of treatment, different parts of the plant are frequently used for multiple purposes. Leaves and roots of \textit{Murraya paniculata} (L.) are stimulant and astringent and are used in the treatment of pain, diarrhoea, dysentery and diseases of teeth and gum; useful against rheumatism, coughs and hysteria [7-9]. Antibacterial and anti-amoebic activities of the plant are also documented [10, 11]. Additionally, it is also used for the treatment of various skin disorders, stomachache and dysentery [12]. The leaves and other tissues are used to treat cuts, joint pain, body aches [13] venereal disease [14] and as an abortive [15]. Phytochemical studies showed the isolation
of alkaloids, prenylated coumarins, polymethoxyflavones and flavonoids [16-18]. Leaves yield oil which contains sesquiterpenes (l-cadinene), a sesquiterpene alcohol and methyl anthranilate [7,8]. In addition to essential oils, tissues of Murraya paniculata contain the indole alkaloid yuehchukene [15] and at least eight highly oxygenated flavones [14].

In the present work, here we reported the isolation of a compound Isomurrayafoline B (Isb). In addition, the isolated compound was tested in established experimental models to evaluate its analgesic activity.

**MATERIALS AND METHODS**

**General Procedure:** Melting point (corrected and uncorrected) was determined in glass capillary tubes with Buchi 535 melting point apparatus and were uncorrected. Infrared spectra were recorded with Perkin Elmer FTIR model 1725X spectrophotometer using KBr discs. The UV spectra were recorded on a Shimadzu UV 240 instrument. Mass spectra were recorded on a VarianMAT 31 double focusing mass spectrometer connected to DEC PDP 11/34 computer system. The NMR spectra were recorded in CDCl₃ on a BrukerAM-300 and AM-400 NMR spectrometers with TMS as internal standard. The optical rotations were measured on a Polatronic Dpolarimeter. Purity of the samples was checked by TLC on silica gel (G-254) pre-coated plates.

**Experimental Animals:** The experiments were carried out using Swiss albino mice (25-30 g) of either sex. All the animals were acclimatized one week prior to the experiments. The animals were kept under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0°C and 12h light-dark cycle) and fed laboratory diet ad libitum and had free access to drinking water. Experiments were performed according to ethical principles established in 1979 for laboratory animals at the service of mankind Lyons, France. All the experiments were conducted on an isolated and noiseless condition.

**Chemicals:** Aspirin (Reckitt and Colman, Pakistan) acetic acid (Sigma chemicals company, St. Louis, USA) Tramadol R (Searle Pakistan Ltd.) Sterile normal saline as control were used in this study.

**Plant Material:** Arial parts of the Plant were collected from District Peshawar of the Khyber Pakhtunkhwa Province and authenticated by plant taxonomist in University WENSAM College, Gomal University Dera Ismail khan. A sample with voucher number GH-040 (2011) was deposited in the Gomal Herbarium of Faculty of Pharmacy Gomal University. After shade drying of aerial parts of the plant for two weeks, it was ground into powder with a laboratory scale mill.

**Extraction and Isolation:** The dried powder of the leaves (13.5 kg) were macerated in methanol (11.5 L) for 48 h and filtered. This process was repeated three times and the combined filtrate was concentrated in vacuo at 40 °C to give crude methanol extract (1.152 kg, 8.53% (w/w). It was redissolved in distilled water and sequentially extracted with hexane, chloroform; ethyl acetate and finally water give the respective fractions. Chloroform fraction (276 g) was subjected to column chromatography and was eluted using chloroform: n-hexane (1:1 to 100:0) gradient on Si gel with gradual increase of polarity to chloroform (100%). This afforded 9 fractions (fr.1-9). Further elution with 1% to 10 % methanol/chloroform gradient has afforded 7 fractions (fr.10-16) fractions. Subfraction 11 (1.29 g) was rechromatographed over Si gel and eluted with 1% methanol-chloroform gradient resulting in 2 subfractions. Further purification by using 1.5 % acetone-chloroform gradient provided Isomurrayafoline B(Isb) (127 mg). Structure of Isomurrayafoline B (Isb) (Figure 1) was recognized via similarity of physical and spectral data with that reported for the corresponding compound in literature [19].

**Analgesic Activity**

**Acetic Acid-induced Writhing:** Acetic acid induced abdominal constriction test [20] was used to investigate the peripheral antinociceptive activity of compounds. In brief, the prescreened animals were divided into 4 groups of six mice each (n = 6). Group 1 used as a control, received normal saline (10 ml/kg, i.p.) and Group 2 (positive control) received standard drug, Aspirin (150 mg/kg p.o.). Test compounds Isb (10 and 20 mg/kg, p.o.) was given to remaining groups. Control, positive control (Aspirin) and test sample were given 30 min prior to injection of 1.0% acetic acid (0.1 ml/10 g body weight; i.p.) which induces writhes in the animals. Abdominal constrictions, that occurred between 5 and 15 min after acetic acid injection were counted for 10 min. A comparison of writhing was made among the groups and percent inhibition of the writhes was calculated by using the formula:

\[\text{Inhibition} (\%) = \frac{\text{Mean number of writhes (control)} - \text{Mean number of writhes (test)}}{\text{Mean no of writhes (control)}} \times 100\]
Thermal Nociception (Hot Plate Test): In this method [21], mice were screened by placing them on a hot metal plate maintained at 50±0.05 °C. In the pretreatment session, mice were tested on two separate occasions, each 30 min apart and mice that showed comparatively similar nociceptive responses within 15 s when placed on the hot plate were admitted into the study and placed into 4 groups of six mice each (n = 6). The first group received normal saline (10 ml/kg), while second and third received Isb, at the dose of 10 and 20 mg/kg, p.o respectively. The last group received Tramadol, an opioid analgesic as a standard drug (20 mg/kg p.o.). Each mouse was gently placed on the hot plate and the time taken by the mice to respond to the thermal stimulus in the form of jumping, withdrawal of the paws or the licking of the paws was recorded. Readings were taken for each mouse at time 0, 30, 60, 90 and 120 min post treatment with a cut off period of 30 s to avoid damage to the paw in the absence of response.

Statistical Analysis: The results obtained were expressed as mean±SEM (Standard error of mean) of six animals. For statistical analysis, one-way ANOVA (independent) was followed by post hoc Dunnett’s test for multiple comparisons. The statistical analyses were carried out using Graph pad Prism version 5.0, software, Inc., USA. Effects were considered to be significant at the P<0.05 level.

Table 1: Effect of the isolated compound on acetic acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Writhing count</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isb10</td>
<td>10 p.o</td>
<td>6.1±0.32**</td>
<td>80.33</td>
</tr>
<tr>
<td>Isb20</td>
<td>20 p.o</td>
<td>1.9±0.38**</td>
<td>93.87</td>
</tr>
<tr>
<td>Aspirin (Positive control)</td>
<td>150 p.o</td>
<td>3.8±0.4**</td>
<td>87.74</td>
</tr>
<tr>
<td>Control (saline)</td>
<td>10 ml/kg</td>
<td>31±1.6</td>
<td>0</td>
</tr>
</tbody>
</table>

Results are expressed as mean±S.E.M. (n= 6); values were compared by ANOVA followed by Dunnett’s test. Significance at* p < 0.05, ** p < 0.01, as compared to control.

Table 2: Effect of the isolated compound on hot-plate test in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(Dose mg/kg)</th>
<th>Reaction time(s) after administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Isb</td>
<td>10 p.o</td>
<td>5.2±0.18</td>
</tr>
<tr>
<td>Isb</td>
<td>20 p.o</td>
<td>5.3±0.15</td>
</tr>
<tr>
<td>Tramadol</td>
<td>20 p.o</td>
<td>5.18±0.15</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>5.29±0.22</td>
</tr>
</tbody>
</table>

Results are expressed as mean±S.E.M. (n= 6); values were compared by ANOVA followed by Dunnett’s test. Significance at* p < 0.05, ** p < 0.01, as compared to the control.
Fig. 1: Chemical structure of isolated compound, Isomurrayafolin B (Isb).

Fig. 2: Decrease in writhing count of Isb (10 and 20 mg/kg p.o.) and Aspirin (150 mg/Kg p.o.) vs Control (saline) in acetic acid-induced writhing test in mice. Each point represents the mean±SEM of 6 animals. The data was analyzed by ANOVA followed by Dunnett’s test. Asterisks indicated statistically significant values from control. * $p<0.05$, ** $p<0.01$.

Fig. 3: Increase in latency time of Isb (10 and 20 mg/kg p.o.) and Tramadol (20mg/kg) on hot plate pain in mice. Each point represents the mean±SEM of 6 animals. The data was analyzed by ANOVA followed by Dunnett’s test. Asterisks indicated statistically significant values from control *$P<0.05$, **$P<0.01$.

The isolated compound exhibited significant analgesic effects in hot plate method, which were similar to that in writhing test, confirming the central activity. Standard drug, Tramadol exerted significant effects after 30 min of administration which remained potent till 120 minutes of administration.

DISCUSSION

The potential nociceptive activity of the compound isolated in the present study was investigated by applying two different analgesic tests, namely cutaneous thermic (hot plate) and chemical visceral (writhing). The results show that isolated compound exerted considerable effects on chemical (Acetic Acid induced) and thermic painful stimuli from the respective doses of 10 and 20 mg/kg.

Writhing is defined as contraction of the smooth muscles of the abdominal region followed by the extension of forelimbs which ultimately ends in elongation of the whole body. The writhing test mediated by acetic acid is the reflection of peripheral pain feeling which is produced due to the release of endogenous inflammatory mediators [22] as well as other pain mediators such as arachidonic acid via cyclooxygenase and prostaglandin biosynthesis [23] which ultimately stimulate the nerve endings. A verity of analgesic drugs, acting peripherally, has inhibited writhing induced by acetic acid in mice [24, 25]. The abdominal constriction is considered to involve local peritoneal receptors [26] and mediated by peritoneal mast cells [27, 28] acid sensing ion channels and the prostaglandin pathways [28]. In mice, intraperitoneal injection of acetic acid liberates prostaglandins such as PGE2 and PGF2; hence their concentration increases in the peritoneal fluid [29]. In the acetic acid induced test, the enhancement in the levels of prostanoids as well as lipoxygenase products have also been observed in the peritoneal fluid [30]. In the present study, significant fall in the writhes was observed for the test compound in acetic acid induced abdominal constriction model which might be due to the inhibition of the synthesis of the arachidonic acid metabolites.

The acetic acid-induced writhing test is lacking specificity and different nociceptive mechanisms may be responsible for the reduction of muscular constrictions such as sympathetic system through the release of biogenic amines, cyclooxygenases and their metabolites inhibition and through opioids receptors mechanisms.
From mechanistic point of view, this compound was also tested in hot plate test to overcome this limitation. Hot plate test is mostly a spinal reflex and mostly used to assess supraspinal analgesia in compounds. The test is therefore considered selective for centrally acting analgesic drugs, like morphine and its analogues, while peripheral antinociceptive agents are found to be inactive on thermal-induced hyperalgesia [31]. Isb showed prominent activity after 60 min of drug administration that was remained significant up to 120 min. In short, the isolated alkaloid from *M. paniculata* provoked outstanding antinociceptive activity in both central and peripheral pain models. Duet effects of these compounds can be characterize as an ideal therapeutic modality in the management of various painful conditions. Whether it involved the mediation of opioid receptors is still a question to be further investigated. It is necessary that other mechanisms responsible for the analgesic effects of isolated compounds need to be understood. Moreover, the isolation of analgesic alkaloids provides scientific foundation to the folkloric uses of the plant as analgesic.

**CONCLUSION**

For the first time, the analgesic activities of the isolated compound, Isomurrayafoline B (Isb) is reported. On the basis of their potent analgesic activity, it could explain and support, in part, the folk use of *M. Paniculata* in traditional medicine. However, more research is required to supplement these findings.

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**REFERENCES**


