Anti-nociceptive and anti-inflammatory activities of *Atalantia retusa* Merr.

Raga Dennis D.* , Cueto Joey C., Ganacias Richard Lester S., and Mandia Emelina H.

Biology Department and Center for Natural Science and Environmental Research Center, College of Science, De La Salle University, 2401 Taft Ave., Manila 1004 Philippines

* Corresponding author. Telefax: +632 536-0228 Email address: dennis.raga@dlsu.edu.ph (DD Raga)

Abstract

*Atalantia retusa* is an endemic medicinal plant used in the Philippines. Hexane extract from leaves were orally administered on rats and mice and tested using rat paw edema, formalin and writhing assays. Increased pain tolerance was observed in animals administered with the median dose (1.43 mg/Kg BW) in both somatic (P<0.05) and visceral (P<0.01) models by 6.05% and 55.48% better compared to the positive control. The degree of swelling was also reduced by the administration of 1.43 mg/Kg BW at 0.5 to 3.0h after carrageenan injection suggesting a high impact analgesic and anti-inflammatory effects of *A. retusa* hexane extract.

Keywords: Analgesic, Anti-inflammatory, Rutaceae, hexane extract.

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*Author for Correspondence: dennis.raga@dlsu.edu.ph

1. INTRODUCTION

Since the earliest times, man has turned to nature for remedies to alleviate pain. Traditional folk medicine usually involves a variety of indigenous plants and herbs for particular illnesses. The Philippines has a rich cultural background complimented with a diverse range of flora. Traditional folk medicine therefore is a common practice in rural areas for people seeking remedy from *herbolaryos* (folk medicine man) for treatment of various illnesses such as toothache, fever, stomach ache, cramps and the like. This has been the basis for the discovery of bioactive compounds that are now being used as prescription drugs.

Pain is usually perceived throughout the body by the numerous specific nociceptors. Analgesic drug relieves pain by blocking pain signals going to the brain or interfering with the brain’s interpretation of signals [1]. Cyclooxygenase inhibitory (COX) drugs inhibit COX-2 thus preventing the conversion of arachidonic acid into prostaglandins formed when membrane bound phospholipids come into contact with phospholipase A2 in the event of trauma or inflammation of tissue [2]. Reduced levels of prostaglandin will also result in the reduction of cytokines such as interleukin-2 (IL-2) thereby desensitizing the CNS and PNS. The desensitized integrating centers therefore will have less sensitivity to pain.

Information on *Atalantia retusa* locally known as *tulan manok* is very limited, although, other species of the Rutaceae family have been studied for its anti-inflammatory, analgesic, antispasmodic and antinociceptive properties. *A. retusa* is a tree growing to a height of about 4-5 meters at an altitude of 130m ASL. It is an endemic species inhabiting the non-teak forests of Mindoro, Palawan, and Panay islands in the Philippines. Locals in Occidental Mindoro claim that the plant is used to treat various ailments among locals. Other members of the Rutaceae family have been documented to have significant antinociceptive and anti-inflammatory properties, but there are no documented studies confirming the antinociceptive and anti-inflammatory properties of *A. retusa*. The current study sought to verify such potential of *A. retusa*.

2. EXPERIMENTAL

2.1 Collection of Plant Material

*Atalantia retusa* leaves from the non-teak forest of Sitio Bunlao, Baranggay Ipil, Ilin Island, San Jose, Occidental Mindoro was collected in April, 2008 and identified by Dr. Emelina H. Mandia with voucher number 893 deposited at the Biology Department, DLSU-Manila.
2.2 Preparation of Plant Extracts

The air-dried leaves (800 grams) were first pulverized and then soaked in 2L Hexane (Ajax FineChem, Australia) for 3 days and then filtered. The filtrate was then concentrated under vacuum to afford 4.47g of non-polar extract. The extract was further liberated from the extracting solvent by desiccation. Polysorbate80 (25%) in corn oil was used as vehicle and kept in cold storage until time of use.

2.3 Animals

Laboratory-bred male ICR Mice (Mus musculus L.) and Sprague-Dawley Rats (Rattus norvegicus) from parent stocks obtained from the Experimental Animal House of the Bureau of Food and Drugs, Muntinlupa City, Philippines were used in the study. A total of 45 male (6-week old) mice, and 70 male (6-week old) rats weighing an average of 30.44±3.74g and 197.03±26.17g, respectively, were acclimatized for 14 days followed by a 2 hour post-acclimatization before the assay proper. The animals were kept in the Animal Containment unit of De La Salle University, under normal conditions with 12 hours daylight and 12 hours darkness, with free access to food pellets (28 %CP, 14% CF) and water. All procedures regarding handling of the test animals were in accordance with the existing guidelines of the Philippine Association of Laboratory Animal Science (PALAS) for care and use of laboratory animals [3] and with Administrative Order 40 of the Bureau of Animal Industry relative to Republic Act No. 8485.

2.4 Antinociceptive activity

2.4.1 Formalin Test [4]

Inhibition of somatic pain was tested on rats (n=6) orally administered with increasing dosages of A. retusa non-polar extract (0.143mg/kg BW, 1.43mg/kg BW and 14.3mg/kg BW) followed by injection of 1% Formalin on the right hind paw 1 hour after administration of the test drug. Paracetamol (10 mg/kg BW, Bristol-Meyers) and 25% Polysorbate80 (25%) were used as positive and negative controls, respectively. The number of bites and paw licks were counted for 10 minutes followed by a second counting period. Total number of scratches and licks are presented as % inhibition. Percent inhibition is computed as 100 – [(Number of bites or scratches per individual/average number of bites or scratches of the negative control) × 100].

2.4.2 Acetic Acid test [5]

Inhibition of visceral pain was tested on mice (n=9) orally administered with Polysorbate80 (25%), Paracetamol (10mg/kg BW), and the non-polar extract of A. retusa, in 3 different dosages followed by an intraperitoneal injection of 1% Glacial Acetic Acid after 1 hour. The number of abdominal stretches completed within 10 minutes were counted and presented as % inhibition.

2.5 Anti-inflammatory activity [6,7]

Male Sprague-Dawley rats (n=8) were orally administered with either nonpolar extract (0.143, 1.43 and 14.3 mg/Kg BW), diclofenac sodium difenax, (1.43 mg/Kg BW, GX International, Philippines) or vehicle (1 ml/ Kg BW) followed by a single plantar injection of 0.1 ml 1% λ-carrageenan (Sigma) at the right foot 1h after oral gavage. The contra-lateral foot was injected with 0.1 ml physiological saline (0.9% NSS) as control. Paw volume was measured plethysmographically before and 0.5, 1, 1.5, 2, 2.5 and 3h after carrageenan injection. The degree of swelling was determined by obtaining the ratio of a/b where a and b are volumes of the same hind paw after and before carrageenan treatment, respectively. A ratio that is smaller than 1.25 indicates a significant inhibitory effect of the extract.

2.7 Statistical Analysis

The results were analyzed using SPSS ver. 13 for Windows. One way Analysis of Variance was performed to determine the significant effects of the analgesic potentials of the A. retusa non-polar extracts. The results were considered significant at P ≤ 0.05. Significant differences between group variables were determined by post hoc analysis at 95% DMRT. Means represent Mean ± SD.

3. RESULTS AND DISCUSSION

3.1 General Observation

The test animals did not show observable indicators of intoxication nor have yielded incidence of mortality brought about by the effects of the extract. This indicates that the dose of the extract tested is non-toxic to the animal which is further confirmed by zero mortality after 24 hours.

3.2 Antinociceptive activity

3.2.1 Formalin test

Male Sprague-Dawley rats received an oral dose of A. retusa non-polar extract, followed by a single injection of 1% formalin to the right hind paw 1 hour after administration of the test drug. The number of paw licks was counted for 10 minutes, followed by a second counting at 30 minutes.
interval. The lowest concentration (0.1437mg/kgBW) and medium concentration (1.437mg/kgBW) of the non-polar extract have shown significant analgesic properties comparable to the effects of Diclofenac (15.67±4.47) evident in the reduced paw licking and biting compared to the negative control (27.00±6.27). The highest concentration of *A. retusa* extract has shown very little analgesic activity (Table 1).

### 3.2.2 Acetic Acid Writhing Assay

Male ICR mice orally administered with increasing dosages of *A. retusa* non-polar extract were given a single injection of 1% Glacial Acetic Acid 1 hour after the administration of the test drug. The number of abdominal stretching was immediately counted for 10 minutes after injection of glacial acetic acid. *A. retusa* non-polar extract have shown significant analgesic effect (P<0.001) at 1.43mg/kg BW and 14.3mg/kg BW which is non comparable with those given the positive control. Mice administered with the median dose of the non-polar extract obtained 90.51±10.05% compared to those mice given Paracetamol 15.67±4.47%, further the non-polar extract was able to surpass the inhibitory effect of Paracetamol by 74.48%. The lowest concentration however (0.0143mg/kg BW) revealed no antinociceptive effect which is not significantly different with the negative control (-0.1±55.46%). (Tab. 1)

### 3.3 Anti-inflammatory activity

*Atalantia retusa* nonpolar extract has significantly reduced inflammatory reaction in λ-carrageenan injected paw. The degree of paw swelling in rats treated with the median dose (1.43 mg/Kg BW) was effectively (P<0.022) reduced by 1.043±0.26 which is not significantly different with the anti-inflammatory activity of Diclofenac (1.08±0.17) at a dose similar to the median dose treatment 1.0h after λ-carrageenan injection. The inhibitory effect of the median dose persisted until 3.0h (Tab. 2) which is still not significantly different with diclofenac. This indicates the positive control and the extract had comparable activity at the dose level tested.

*Means followed by the same letter is not significantly different at 95% DMRT.(0.05α)

### Table 1 Somatic and Visceral Pain Models

<table>
<thead>
<tr>
<th>Somatic Model (Formalin Test)</th>
<th>Visceral Model (Writhing Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of Paw Licks</strong></td>
<td><strong>% Inhibition</strong></td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>15.67±4.41</td>
</tr>
<tr>
<td>25% Polysorbate80</td>
<td>22.00 ± 6.26</td>
</tr>
<tr>
<td>0.1437 mg/kg BW</td>
<td>15.5 ± 4.09</td>
</tr>
<tr>
<td>1.437 mg/kg BW</td>
<td>14.33 ± 3.14</td>
</tr>
<tr>
<td>14.37 mg/kg BW</td>
<td>18.67 ± 3.78ab</td>
</tr>
</tbody>
</table>

*Means followed by the same letter is not significantly different at 95% DMRT.(0.05α)*

### Table 2 Degree of swelling in rat hind paw.

<table>
<thead>
<tr>
<th>Right Paw (λ-carrageenan)</th>
<th>0.5h</th>
<th>1.0h</th>
<th>1.5h</th>
<th>2.0h</th>
<th>2.5h</th>
<th>3.0h</th>
</tr>
</thead>
<tbody>
<tr>
<td>25% Polysorbate80</td>
<td>1.36 ± 0.38</td>
<td>1.33 ± 0.35</td>
<td>1.52 ± 0.39</td>
<td>1.54 ± 0.33</td>
<td>1.48 ± 0.34</td>
<td>1.56 ± 0.45</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1.14 ± 0.13</td>
<td>1.08 ± 0.17ab</td>
<td>1.27 ± 0.30</td>
<td>1.31 ± 0.19</td>
<td>1.23 ± 0.02a</td>
<td>1.14 ± 0.13a</td>
</tr>
<tr>
<td>0.143 mg/kg BW</td>
<td>1.28 ± 0.28</td>
<td>1.37 ± 0.33</td>
<td>1.64 ± 0.36</td>
<td>1.59 ± 0.24</td>
<td>1.67 ± 0.36</td>
<td>1.59 ± 0.45</td>
</tr>
<tr>
<td>1.43 mg/kg BW</td>
<td>1.06 ± 0.19</td>
<td>1.04 ± 0.26</td>
<td>1.23 ± 0.32</td>
<td>1.31 ± 0.33</td>
<td>1.26 ± 0.39</td>
<td>1.20 ± 0.40</td>
</tr>
<tr>
<td>14.37 mg/kg BW</td>
<td>1.12 ± 0.17</td>
<td>1.43 ± 0.23</td>
<td>1.68 ± 0.22</td>
<td>1.72 ± 0.33</td>
<td>1.68 ± 0.22</td>
<td>1.43 ± 0.18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Left Paw (0.9% Saline)</th>
<th>0.5h</th>
<th>1.0h</th>
<th>1.5h</th>
<th>2.0h</th>
<th>2.5h</th>
<th>3.0h</th>
</tr>
</thead>
<tbody>
<tr>
<td>25% Polysorbate80</td>
<td>1.08 ± 0.17ab</td>
<td>1.18 ± 0.20</td>
<td>1.25 ± 0.18</td>
<td>1.25 ± 0.13</td>
<td>1.21 ± 0.18</td>
<td>1.18 ± 0.14</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>1.21 ± 0.16a</td>
<td>1.01 ± 0.12</td>
<td>1.22 ± 0.28</td>
<td>1.23 ± 0.26</td>
<td>1.29 ± 0.21</td>
<td>1.19 ± 0.27</td>
</tr>
<tr>
<td>0.1437mg/kg BW</td>
<td>1.23 ± 0.23b</td>
<td>1.12 ± 0.17</td>
<td>1.29 ± 0.23</td>
<td>1.23 ± 0.26</td>
<td>1.39 ± 0.35</td>
<td>1.16 ± 0.25</td>
</tr>
<tr>
<td>1.437mg/kg BW</td>
<td>0.98 ± 0.06c</td>
<td>1.01 ± 0.11</td>
<td>1.00 ± 0.17</td>
<td>1.16 ± 0.10</td>
<td>1.15 ± 0.25</td>
<td>1.02 ± 0.17</td>
</tr>
<tr>
<td>14.37mg/kg BW</td>
<td>0.94 ± 0.17a</td>
<td>1.09 ± 0.13</td>
<td>1.14 ± 0.20</td>
<td>1.20 ± 0.18</td>
<td>1.13 ± 0.19</td>
<td>1.21 ± 0.26</td>
</tr>
</tbody>
</table>

*Means followed by the same letter is not significantly different at 95% DMRT.(0.05α)*
pronounced during the early phase of inflammatory reaction suggesting the central neurologic action of the extract. Injection of physiological saline in the left paw indicates that the subplantar injection had minimal involvement in the inflammatory reaction in the test animals confirming that the inflammation was due to carrageenan and the inhibition of inflammation was due to the activity of the extract. The lowest dose (0.143 mg/Kg BW) revealed no inhibition of paw swelling particularly suggesting that the dose is too low to demonstrate pharmacologic effect while the highest dose (14.3 mg/Kg BW) is too high that the maximum effective dose has been reached that no obvious pharmacologic effect is observed.

3.4 Discussion

Overall, the non-polar extract from *A. retusa* leaves has shown significant analgesic effect, which has been observed to be more effective than paracetamol or diclofenac. The complementary action of analgesic and the anti-inflammatory activity in known test drugs such as paracetamol and diclofenac was significantly demonstrated in the experimental animals treated with median dose *A. retusa* nonpolar extract with no significant differences with the positive control. The data suggest that such dose is as effective as the commercial dosages of diclofenac and paracetamol. The intraperitoneal injection of 1% acetic acid induced unpleasant stimuli observed from the writhing response of mice similar to rats subcutaneously injected with formalin. It is postulated that the release of PGE2 and PGF2α [8,9] along with TNF-α [10] may have been indirectly induced by irritant in peritoneal fluid and subplantar tissue of both positive control and *A. retusa* extract treated mice that may have possibly reduced COX enzyme activity [11] similar to the action of common non-selective COX inhibitors which is believed to be acting on lipooxygenase and/or cyclooxygenase in peripheral tissues [12]. Acute inflammation triggered by the specific action of λ-carrageenan promotes the accumulation pro-inflammatory factors such as prostaglandin which are important mediators of inflammatory reactions [13,14]. The synthesis of inflammatory mediators may have been possibly inhibited by limiting the production of necessary mediators to produce specific inflammatory responses [15]. The possible reduction of inflammatory mediator synthesis may be responsible for the non-stimulation of nociceptors. It is hypothesized that the possible inhibition of COX is involved in mediating peripheral neurologic aspects [12] of pain demonstrated in the visceral model and both peripheral and central neurologic desensitization [16] exhibited in the somatic model and inflammation may have been the possible mechanism involved. Further test, however and should be conducted to confirm such mechanism of action.

Rutecarpine, a non-polar indolopyridoquinazolinone alkaloid isolated from *Evodia rutaecarpa* (Rutaceae) was documented to have anti-inflammatory and analgesic properties [11] which has been tied to its ability to inhibit COX-2 and prostaglandin synthesis [17]. It is most likely that *A. retusa* may also contain rutecarpine, but further studies on isolation and purification of the extract is recommended since the major sources of this compound are members of the Rutaceae family such as *Hortia*, *Zanthoxylum*, *Phellodendron*, *Tetradium*, *Spiranthera*, *Vepris*, *Metrodorea*, *Bouchardatia*, and *Fagara*. [11,18,19,20,21,22].

4.0 CONCLUSION

The current study presents the analgesic and anti-inflammatory property of *Atalantia retusa* non-polar extract in mice and rats, respectively. In the somatic pain model, the median dose of the extract has demonstrated significant analgesic property acting centrally and peripherally which has surpassed the anti-nociceptive effect of Paracetamol (10mg/kg BW) and equaled the anti-inflammatory effect diclofenac (1.43 mg/Kg BW). Potency of the extract, however, seems to diminish at the highest dose tested, which is concomitant with the effects observed in the visceral model demonstrating the maximum dose effect. The findings indicate the dose 1.437 mg/Kg BW to be the most effective dose comparable to the positive control acting peripherally which has surpassed the anti-nociceptive effect of Paracetamol (10mg/kg BW) and equaled the anti-inflammatory effect diclofenac (1.43 mg/Kg BW). The anti-inflammatory activity diclofenac (1.43 mg/Kg BW) is too high that the maximum effective dose has been reached that no obvious pharmacologic effect is observed.

REFERENCES


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