EVALUATION OF THE ANALGESIC PROPERTIES OF THE METHANOL ROOT EXTRACT OF *ANTHOCLEISTA DJALONENSIS* A.CHEV

Hope Delesi Kagbo¹* and Sandra Ebiere Simon²

¹Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria.

²Department of Biomedical Technology, School of Science Laboratory Technology University of Port Harcourt, Nigeria.

ABSTRACT

The root extract of *Anthocleista djalonensis* A.Chev. was prepared by maceration in methanol and the extract evaporated to dryness. The extract was investigated for analgesic properties in albino rats using chemical, mechanical and thermal models of pain. The formalin hind paw licking test which evaluates the response to neurogenic and inflammatory pains was used to study chemically induced pain. The animals were observed for paw licking for thirty minutes following subcutaneous administration of buffered formalin in the hind paw. Mechanical pain was induced by exertion of pressure on inflammed and hyperalgesic rat paw with the Analgesy-meter (UgoBasile Model No. 37215). The supraspinally mediated tail flick test, where the time it takes for the rat to withdraw its tail from an environment of adverse extreme of temperature is observed, was used to assess the effect of the extract on thermally induced pain. In all these models, the extract showed a significant (p<0.05 - 0.001), dose dependent inhibition of nociception. The result from this study therefore suggest that the extract act at central and peripheral sites to inhibit neurogenic and inflammatory pains.

KEYWORDS: *Anthocleista djalonensis*, Randall-Selitto method, Tail Immersion Test, Formalin test.
INTRODUCTION
Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine. There has been a tremendous resurgence of interest in recent years in the use of medicinal plant products for the control of diverse ailments, because they are thought to be safer, more effective, inexpensive and superior to that of synthesized compounds.\(^1\)

*Anthocleista djalonensis* A.Chev., a medium sized tree found mostly in West Africa, Comoros and Madagascar is used traditionally for the treatment of various disease, the plant is known for its antipyretic, stomachic, analgesic and purgative actions.\(^2\) Herbalists claim a high percentage of cures in their diabetic patients treated with it.\(^3\) The leaves are reputed to be used for the treatment of malaria and jaundice.\(^4\) The bark is used as a purgative in small doses as large doses are considered toxic. According to the Mendi ethnomedicine, when the tree is used as firewood, the people sitting around it becomes sick.\(^5\) A root decoction is commonly taken to treat constipation to regulate menstruation and as an abortifacient.\(^6,7\)

MATERIALS AND METHODS

Plant Collection and Identification

The root sample of *Anthocleista djalonensis* was harvested from Alakahia, Rivers State, Nigeria and was identified and authenticated by Dr N. L. Edwin-Wosu, a Taxonomist at the Herbarium of University of Port Harcourt, Nigeria. Pictorial and Voucher samples were deposited at the herbarium with reference number (UPH / NO-P-053) For Further Studies.

Extraction Procedures

The roots of *Anthocleista djalonensis* were washed immediately after uprooting and chopped into smaller bits and kept in a well ventilated room and allowed to dry at room temperature for three weeks. The dried roots were reduced to a fine powder with a mechanical grinder, filtered, and the fine powder was stored in a polyethylene bag. The pulverized substance was macerated in methanol for 72 hours and stirred, thereafter, it was filtered with a Millipore and the filtrate was evaporated to dryness using rotary evaporator. The dry powder was kept at room temperature and used for the study.

Animal Handling

Wistar albino rats weighing 200 - 320g of both sexes from Department of Pharmacology, University of Port-Harcourt animal house were used for this study. The animals were maintained under standard environmental conditions. The animals were provided with
standard rodent pellet diet (finisher feed) and water, *ad libitum*. Food was withdrawn 24 hours before the experiment was conducted. The care and handling of these animals were carried out in strict compliance with the current guidelines of the International Association for the Study of Pain, for the use of animals in pain research. [8]

**Experimental Procedures**

**Formalin-Induced Hind Paw Licking:**

The effect of the extract on chemically induced pain was investigated with the formalin hind-paw licking model.[9]

The animals were separated into five groups of five animals each. Groups 2-4 received 200, 400 and 800mg/kg of the extract respectively. Group 5 was administered with 40mg/kg indomethacin, while Group 1 served as control and was given 10ml/kg normal saline. After thirty minutes, all the animals were challenged with 0.1ml buffered formalin, subcutaneously under the surface of the right hind paw and individually placed in transparent observation chambers. The rats were observed for 1 hour after the injection of formalin, and the amount of time spent licking the injected paw was recorded and considered as indicative of pain.

**Randall Sellitto Method**

In this study the Randall-Selitto method was adopted for assessment of the effect of the extract on mechanically induced pain.[10,11]

The method was carried out using the Analgesy-meter (UgoBasile Model No. 37215). The animals were separated into five groups of five animals each. Groups 2-4 received 200, 400 and 800mg/kg of the extract respectively. Group 5 was administered with 40mg/kg indomethacin, while Group 1 served as control and was given 10ml/kg normal saline. Thirty minutes later, each animal was administered with 0.1ml of egg albumen subcutaneously into the sub-plantar of the right hind paw. After thirty minutes of administration of the egg albumen, the animal’s inflamed paw is placed on a plinth and the cone-shaped pusher with a rounded tip of the Analgesy-meter was used to apply a force to the paw until the animal struggles to remove its paw from the plinth. This is indicative of pain. At this point, the reading on the Analgesy-meter was taken.
Tail Immersion Test

Thermally induced pain was evaluated with some modifications to the tail immersion test.\[12,13\] An ice cold water tail immersion method was used instead of warm water to evaluate the supraspinally mediated analgesic effect of *A. djalonensis* extract on rats.

Animals in group 2 to 4 were pre-treated with various doses of the plant extract, group 5 was pre-treated with indomethacin, while Group 1 served as control. All the substances were administered intraperitoneally. After 30 minutes of administration, the lower two-third of the rat tail in the various groups were immersed in cold water maintained at a temperature of 4 ± 2°C and timed with a stop watch.

The time taken before the animal react to the cold sensation (pain) (which is the time between the immersion of the tail and withdrawal of the tail by the rat) was taken as a measure of nociception and recorded as the reaction time.

**3.5 Statistical Analysis**

The data obtained were analyzed using the Graphpad Prism version 6.01. The values from the test doses were compared with the control using One Way Analysis of Variance (ANOVA) followed by Dunnet’s Post Test.

**RESULTS**

Effect of extract on chemically-induced pain

Formalin-induced hind paw licking in rats

The extract showed significant (p<0.01–0.001) reduction in hind paw licking (analgesic effect) in the early phase (first 5 min) relative to control. There was also late phase reduction in hind paw licking.

**Table 1: Effect of extract on formalin-induced hind paw licking in rat.**

<table>
<thead>
<tr>
<th>TIME =DOSE (mg/kg)</th>
<th>5min</th>
<th>10min</th>
<th>15min</th>
<th>20min</th>
<th>25min</th>
<th>30min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.80±5.49</td>
<td>39.40±8.54</td>
<td>25.40±8.83</td>
<td>8.600±4.09</td>
<td>17.00±11.14</td>
<td>28.60±11.81</td>
</tr>
<tr>
<td>200</td>
<td>16.8±7.05**</td>
<td>12.6±3.27**</td>
<td>12.4±7.09**</td>
<td>11.4±4.67**</td>
<td>9.2±3.81**</td>
<td>6.2±2.31**</td>
</tr>
<tr>
<td>400</td>
<td>16.2±2.478**</td>
<td>12.4±2.462**</td>
<td>12.0±4.970**ns</td>
<td>9.8±5.678**fsns</td>
<td>8.6±4.729**fsns</td>
<td>5.8±3.555**</td>
</tr>
<tr>
<td>800</td>
<td>12.40±4.49**</td>
<td>7.20±2.26**</td>
<td>6.80±2.42**fs</td>
<td>6.40±3.53**fs</td>
<td>8.60±4.735**fsns</td>
<td>0.60±0.60**fs</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>23.40±3.75**</td>
<td>20.00±3.86**</td>
<td>19.80±5.43**fs</td>
<td>9.00±1.87**fsns</td>
<td>4.60±2.14**fsns</td>
<td>2.80±1.32**</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM.  
ns = Not significant  
** = P<0.01  
*** = P<0.001  
* = P<0.05
Effect of extract on mechanically-induced pain

Egg albumin induced hyperalgesia

The extract showed a significant (p<0.05-0.001), dose-dependent antinociceptive effect against hyperalgesia. The analgesic effect was superior to that of indomethacin, the reference drug.

Fig 1: Effect of extract on mechanically-induced pain (Randall-Sellito method).

Values are expressed as Mean ± SEM.

ns = Not significant
* = P<0.05
** = P<0.01
*** = P<0.001

Effect of extract on thermally-induced pain

Effect of Extract on Tail-Immersion Test in rats

The result showed that there was a significant (P<0.05 – 0.001), dose-dependent increase in reaction time of the treated animals relative to control as shown in Figure 2 below.

Figure 2: Effect of extract on tail immersion test in rats

Values are expressed as Mean ± SEM.

ns = Not significant
* = P<0.05
** = P<0.01
*** = P<0.001
DISCUSSION

The analgesic effect of *Anthocleista djalonensis* was evaluated using chemically, mechanically and thermally induced pain models. The extract was found to significantly (p<0.05 – 0.001) inhibit nociception in all the pain models.

The formalin hind-paw licking test in the rats is sensitive to non-steroidal anti-inflammatory drugs and other mild analgesics. Formalin produces a distinctive biphasic response. The first of the nociceptive response (the early phase) normally peaks 5min after injection and the second phase (the late phase) 15-30min after formalin injection. The early phase corresponds to acute neurogenic pain which reflects directly the effect of formalin on nociceptors and is sensitive to drugs that interact with the opioid system whereas the late phase corresponds to inflammatory pain responses and inhibited by analgesic and anti-inflammatory drugs. \(^{[14]}\) The injection of formalin has been reported to cause an immediate and intense increase in the spontaneous activity of C afferent fiber and evoke a distinct quantifiable behavior indicative of pain demonstrated in paw licking by the rats. \(^{[15]}\) Thus the nociceptive effect of *A. djalonensis* on the first phase of formalin test proposes its central action. The protective effect of *A. djalonensis* on the second phase of formalin test is due to anti-inflammatory effect on peripheral tissue which explains the antinociceptive effect in the second phase.

The extract caused a dose and time-dependent antinociception against mechanically and chemically-induced (nociception) pain in the albino rats.

Fresh egg albumin-induced hyperalgesia involves three distinct phases of mediators release including histamine and 5-hydroxytryptamine in the first phase, kinins in the second phase and prostaglandin in the third phase. \(^{[16,17]}\) Prostaglandin in particular, is known to cause or enhance the cardinal signs of inflammation. \(^{[17]}\)

The extract progressively reduced hyperalgesia induced by these chemicals. Due to a primary stimulus, two mechanisms contribute to the development of hyperalgesia caused by increased vascular permeability. One induced by local release or formation of various autacoids and another induced neurogenically by stimulation of primary sensory neurons and subsequent mediator (substance P) released from peripheral endings of these fibers. \(^{[18,19,20]}\)

The neurogenic component plays an important role in maintaining the non-neurogenic plasma extravasation since the stimulation of peripheral neurons and subsequent release of substance
P from peripheral sensory endings causes further release of histamine from mast cells. It therefore means that the possible specific action of this extract in blocking the neurogenic component of the stimulated vascular permeability can stop the series of pathogenic events locally evoked by noxious stimuli.\(^{[21]}\)

The anti-nociceptive activities exerted by this extract may be attributed to the presence of secondary metabolites like saponins, flavonoids, tannins, reducing sugar, steroids, phlobatanins, volatile oils and alkaloids. Flavonoids also have analgesic and anti-inflammatory effects through its inhibition of cyclooxygenase pathway.\(^{[22]}\) That the extract inhibited neurogenic and non-neurogenic pains may in part explain the mechanisms of its action and these effects are due to the presence of phytochemical components in the extract.

The result also showed that the extract inhibited mechanical pain in the rats. The paw pressure test is a model, widely used for quantification of thresholds of the rat hind paw withdrawal reflex to the nociceptive pressure mechanical pain in the rats. Pain mediators like cyclooxygenase products (PGE\(_2\)) leukotrienes, cytokines (IL-I\(\beta\) and TNF\(\alpha\)), nitric oxide, nerve growth factor (NGF), and transcription factors (nuclear factor kappa B; NF-\(\kappa\)B)\(^{[23]}\) are released as a result of tissue injury.

Most of these mediators (especially prostaglandins) sensitize peripheral nociceptors to noxious stimuli and subsequently release mediators in the spinal cord, resulting in mechanical hyperalgesia.\(^{[24]}\) Activation of the transcription factor NF-\(\kappa\)B has been shown to be a key component in the expression of genes involved in the production of inflammatory mediators, which may exacerbate pain, hyperalgesia, and nociception.\(^{[25]}\)

Therefore, the effectiveness of the extract against mechanical pain in this model suggests that this extract may be acting against of some these mediators. The result obtained corroborate the observed activity of the extract in the second phase (inflammatory pain) of the formalin test.

Analgesic drugs are usually classified based on their mechanism of action, either on the central nervous system or on the peripheral nervous system.\(^{[26]}\) Tail immersion test is supraspinally mediated and therefore a test for central activity. It has been found to be suitable for evaluation of centrally acting analgesics.\(^{[27]}\) The noceceptors seemed to be sensitized by sensory nerves and the involvement of endogenous substances such as prostaglandins may be minimized in this model of analgesic.
A. djalonensis in this study demonstrated central action by increasing the reaction time to cold. It is an established fact that any agent that causes a prolongation of tail immersion latency using this test must be acting centrally.

CONCLUSION

The results from this study has demonstrated that the root extract of Anthocleista djalonensis possesses analgesic property. Although the exact mechanism of analgesic activity is not known, the inhibition of nociception in the chemical, mechanical and thermal models of pain suggests that the extract acts at central and peripheral sites to produce its analgesic effects.

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