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Phytochemical Investigation and Evaluation of Analgesic Effect of Ethanolic Leaves Extract of *Loranthus micranthus* Linn (Nigerian Mistletoe)

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Abstract

Pain has been affirmed as the most common symptomatic presentation in ailment. Increasing number of recent studies on welfare and health status have been aimed at retrieving analgesic agents from natural sources. The aim of this study was to evaluate the analgesic effect of the ethanolic leaves extract of *Loranthus micranthus* Linn (Mistletoe) in wistar rats. The leaves of the plant were extracted using 95% ethanol and the extract obtained evaluated for its analgesic activity using both the hot plate and acetic acid induced writhing methods. The effect of the extract (at all tested doses) on the latency of nociceptive response in rats using the hot plate method showed no significant difference when compared with the negative control group. However, there was a significant dose dependent decrease in the number of abdominal writhes in the rats when compared with the negative control ($P<0.05$). At the tested doses of 600 mg/kg, 300 mg/kg, and 150 mg/kg, percentage inhibition of writhing was found to be 66%, 59%, and 12% respectively. it is therefore conceivable that the ethanolic leaves extract of *Loranthus micranthus* Linn possesses peripheral analgesic property. However, further studies are necessary in order to isolate and characterize the active principles of the plant as well as further analgesic testing.

Keywords: Analgesia; Ethanolic extract; Pain; *Loranthus micranthus* Linn; Acetic acid induced writhing

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Introduction

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. An analgesic is a drug that relieves pain. Mistletoes are semi-parasitic flowering plants with over 1300 species belonging to the polyphyletic group [1]. *Loranthus micranthus* Linn belonging to the family of African mistletoe; Loranthaceae, is the most common specie of mistletoe found growing on trees and shrubs in Nigeria [2]. Mistletoe has been reported to possess numerous beneficial ethnomedical properties including mitigation of migraine, menopausal syndrome, rheumatism, hypertension, infertility, diabetes, cancer, epilepsy and antimicrobial potential [2,3]. The analgesic effect of ethanolic leaves extract of *Loranthus micranthus* Linn obtained from Nigeria using acetic acid induced writhing in rats is yet to be conducted. This study is therefore aimed at evaluating

the analgesic effect of the ethanolic leaves extract of *Loranthus micranthus* Linn in wistar rats.

Materials and Methods

Source of plant material, collection and authentication

The leaves of *Loranthus micranthus* Linn were collected in the month of February 2017 from Agba, Osun State, Nigeria and was identified and authenticated by botanists the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. A voucher specimen number was assigned (2961) and a sample deposited in the Department.

Preparation of the leaf extracts

The leaves were air dried at room temperature for seven days and ground into a powder using a wooden pestle and mortar. The powdered plant material was then stored in an airtight container prior to extraction. Five hundred grams of the powder was weighed out and defatted using petroleum ether. It was then subjected to series of maceration using 300 ml of 95% ethanol and the extract decanted at an interval of 24 hours within 72 hours. It was then subjected to the rota-vapour machine in order to recover the ethanol and obtain the ethanolic extract which gives a brownish sticky residue with a yield of 37.43 g (7.48%).

Experimental animals

Adult Wistar rats of both sexes, weighing between 120 g to 160 g were purchased from the animal house unit of the Faculty of Pharmacy, University of Maiduguri, Borno State, Nigeria. The animals were maintained in wire mesh cages in the animal section, Pharmacology and Toxicology Laboratory, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, Borno State, Nigeria. Adequate time was given to the animals to acclimatize with the laboratory conditions. The animals were fed with standard pellets made from commercial growers and water was provided ad-libitum. The experimental procedures were conducted in accordance with the guideline on the use of laboratory animals [4].

Phytochemical test: Phyto chemical screening for alkaloids, cardenolides, anthraquinone, saponins, tannins, flavonoids and carotenoids were carried out on the extract according to the methods described by Sofowora, Trease and Evans [5-7].

Test for alkaloids: One gram of extract was dissolved in 5 ml of water and Dragendoffs reagent was added. The presence of orange red precipitate indicates the presence of alkaloids.

Test for ardenolides: One gram of extract was dissolved in 2 ml of glacial acetic acid containing one drop of $FeCl_3$ solution. The mixture was then poured into a test tube containing 1 ml of concentrated H_2SO_4. A brown ring at the inter-phase indicates the presence of a deoxy sugar, characteristic of cardenolides.

Test for anthraquinone: To test for anthraquinone, 0.5 g of the extract was mixed with 5 ml of ferric chloride and 5 ml dilute hydrochloric acid in a test tube. It was then boiled on a water bath for 5 minutes. It was cooled and dilute ammonia about half of its volume was added and shaken. The absence of colouration indicates the absence of anthraquinones.

Test for saponins: Exact 0.5 g of the extract was dissolved in distilled water in a test tube. The absence of persistent froathing on warming was taken as preliminary evidence for the absence of saponins.

Test for tanins: Five drops of ferric chloride solution was added to 1 g of extract contained in a test tube and mixed. A blue black colour indicates the presence of tannins.

Test for flavonoids: Exact 5 ml of dilute ammonia solution was added to a portion of the extract followed by the addition of concentrated H_2SO_4. A yellow colouration was observed which indicates the presence of flavonoids.

Test for carotenoids: Two drops of saturated $SbCl_3$ in $CHCl_3$ was added to 1 g of the extract in a test tube. A blue-green colour eventually changing to red indicates the presence of carotenoids.

Assay of analgesic activity of the extract using hot plate method

Evaluation of analgesic activity using hot plate method was carried out in 30 wistar rats (5 groups, each consisting of 6 rats). Group 1 and 5 served as negative (distilled water) and positive (pentazocine) controls respectively. Group 2, 3 and 4 received 600 mg/kg, 300 mg/kg and 150 mg/kg intraperitoneally of the extract respectively. Group 5 received a standard dose of pentazocine intraperitoneally (10 mg/kg). The rats were placed singly on a hot plate maintained at 55 ± 0.5°C thirty minutes after administration of the extract or drug. Time taken for responses like paw licking or jumping to occur was recorded according to the method described.

Assay of analgesic activity of the extract using acetic acid induced abdominal writhing method

Assaying analgesic activity using the abdominal writhing method was also carried out in 30 wistar rats (5 groups, each consisting of 6 rats). Groups 1 and 5 served as negative (2 mL/kg distilled water) and positive (acetyl salicylic acid) controls respectively. While groups 2, 3 and 4 received doses of 600 mg/kg, 300 mg/kg and 150 mg/kg intra-peritoneally of the extract respectively. Group 5 received acetyl salicylic acid (aspirin) 2 mg/kg intra-peritoneally.

The rats were then injected intra-peritonealy with a 0.2 ml of 3% acetic acid solution, thirty minutes after treatment with the extract or acetyl salicylic acid (aspirin), which induced the characteristic writhing. The number of writhes was observed for 5-15 minutes. The data was collected and computed according to the following formula as described by [8].

$$Percentage\ inhibition\ of\ writhing = \frac{Mean\ number\ of\ writhing\ (negative\ control) - Mean\ number\ of\ writhing\ (test)}{Mean\ number\ of\ writhing\ (negative\ control)} \times 100$$

Statistical analysis

Data obtained from this study was expressed as mean ± standard error of the mean (SEM) and analysed by one-way analysis of variance (ANOVA) using GraphPad prism. The statistical

Table 1 Effect of ethanolic leaves extract of *Loranthus micranthus Linn* on the latency of nociceptive response in rats using hot-plate method.

Sample	Alkaloid Cardenolides	Anthraq	Saponins	Tannins	Flovo	Carote
L. micranthus	+	-	-	+	+	+

Anthraq=Anthraquinones; Flavo=flavonoids; Carote=carotenoids; Present +; Absent -

Table 2 Effects of ethanolic leaves extract of *Loranthus micranthus Linn* on the latency of nociceptive response in rats using hot-plate method.

Treatment	Dose	Latency of Nociceptive Response (sec)
Control	2 mL/kg	1.37 ± 0.07
Pentazocine	10 mg/kg	3.72 ± 0.11
L. micranthus extract	600 mg/kg	1.35 ± 0.08
L. micranthus extract	300 mg/kg	1.37 ± 0.05
L. micranthus extract	150 mg/kg	1.36 ± 0.09

Table 3 Effect of ethanolic leaves extract of *Loranthus micranthus Linn* on acetic acid induced abdominal writhing in rats

Treatment	Dose	Mean No of Writhing	% Inhibition of Writhing
Control	2 mL/kg	33.25 ± 0.47	-
Aspirin	2 mg/kg	14.50 ± 0.28*	56
L. micranthus extract	600 mg/kg	11.25 ± 0.24*	66
L. micranthus extract	300 mg/kg	13.50 ± 0.28*	59
L. micranthus extract	150 mg/kg	29.25 ± 0.25*	12

*(P<0.05) compared with control; all values are expressed as mean ± standard error of the mean (n=6)

significance of differences was estimated by the student t-test and values of P<0.05 were considered to be significant.

Results

Phytochemical studies

The phytochemical screening of the ethanolic leaves extract of *Loranthus micranthus* Linn revealed the presence of alkaloids, cardenolides, tannins, flavonoids and carotenoids **(Table 1).**

Effect of ethanolic leaves extract of *Loranthus micranthus* Linn on the latency of nociceptive response in rats using hot-plate method

The reference drug (pentazocine) conferred significant protection against the nociceptive stimuli, while the extract (at all tested doses) did not show any increase in latency of nociceptive response **(Table 2).**

Effects of ethanolic leaves extract of *Loranthus micranthus* Linn on acetic acid induced abdominal writhing in rats

The reference drug (aspirin) significantly decreased writhing after acetic acid administration. The extract equally showed a dose dependence effect in decreasing the number of abdominal writhes in the test subjects **(Table 3).**

Discussion

Pain has been associated with the pathophysiology of various clinical conditions such as arthritis, multiple sclerosis, colitis, vascular diseases, inflammatory bowel disease cancer, asthma, and atherosclerosis. The phytochemical screening of the ethanolic leaves extract of *Loranthus micranthus* showed the presence of alkaloids, cardenolides, tanins, flavonoids and carotenoids which is similar to the findings of [9] while saponins and anthraquinones were found to be absent in this leaves sample. The variation obtained in this study may be attributed to the difference in geographical location and other environmental factors [10]. Results of the present study shows that leaves extracts of *Loranthus micranthus* have marked analgesic activities thus disclosing the effectiveness of the plant in folk medicine [11]. The results of this study corroborates the work of Haque [12] who reported that *Loranthus micranthus* leaves extract possessed potent analgesic properties when used in mice. This property was attributed to the alkaloid and flavonoid content contained in the leaves of the plant [12]. Acetic acid-induced writhing is a reliable protocol used in the evaluation of analgesic properties of medicinal agents [13]. Analgesics can either act on the peripheral or central nervous system. Peripherally, analgesics block generation of impulses at pain chemoreceptors sites, while centrally, analgesics raise the threshold for pain, and alter biological response to pain; suppressing the patient's apprehension and nervousness [14]. Abdominal writhing is connected with local peritoneal receptor owing to the activation of acid-sensitive ion channels (ASICs) and transient receptor potential vanilloid-1 (TRPV1) confined in afferent primary fibers [15]. Acetic acid causes incendiary pain by inducing capillary permeability and releasing endogenous substances that stimulate pain nerve ending, where the pain sensation originates by generating localized inflammatory reaction due to release of free arachidonic acid from cellular phospholipids via cyclo-oxygenase and producing prostaglandin specifically PGE2 and PGF2α. The level of lipoxygenase products may also increase in peritoneal fluids [16]. More specifically, acetic acid injection induces a release of TNF-α, interleukin-1β (IL-1β) and interleukin-8(IL-8) by resident peritoneal macrophages, mast cells, prostanoids and bradykinin [15]. These prostaglandin and lipoxygenase products are responsible for inflammation and pain. Substance(s) inhibiting the writhing response will have analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [17]. Non-steroidal anti-inflammatory drugs (NSAIDs) can inhibit COX in peripheral tissues and interfere with the mechanism of transduction of primary afferent nociceptors. The mechanism of analgesic activity of leaves extracts *Loranthus micranthus* is probably due to the blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of Morphine sulfate and NSAIDs. Thus, the statistically significant (P<0.05) reduction in the number of writhing indicates that it might exert analgesic

activity by inhibition of prostaglandin synthesis or by action on prostaglandin. The effect of the extract on the latency of nociceptive response in wistar rats using hot plate method at all tested doses did not show any significance difference when compared with the negative control group (distilled water). On the other hand, pentazocine (positive control) was found to increase the latency of nociceptive response significantly ($P<0.05$). This agrees with the findings of [18]. Thermal stimuli are selective for the evaluation of centrally, but not peripherally acting analgesic drugs [19]. Acetic acid-induced nociception is usually used for the evaluation of mild peripheral analgesic and nonsteroidal anti-inflammatory compounds [20,21]. The effects of the ethanolic leaves exract of *Loranthus micranthus* on acetic acid induced abdominal writhing in rats showed a dose dependent significant decrease in the number of abdominal writhes compared with the negative control ($P<0.05$). The positive control (aspirin) also showed a significant decrease in the number of abdominal writhes ($P<0.05$) similar to that of the extract (300 mg/kg). It can therefore be inferred that the ethanolic leaves exract of *Loranthus micranthus* possesses peripheral analgesic properties.

Conclusion

This study showed that the ethanolic leaves exract of *Loranthus micranthus* in wistar rats (at doses tested) exhibited peripheral analgesic activity. The presence of some bioactive constituents such as alkaloids and flavonoids in the leaves may be responsible for the observed effect. These results further support the traditional use of this plant in medicine. However, further studies and phytochemical analysis are necessary in order to isolate and characterize the active principles of *Loranthus micranthus* so as to better understand the mechanisms of action and exploit this plant for future drug development.

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