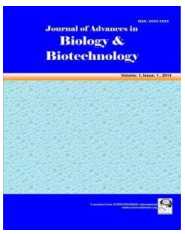

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Analgesic (Antinociceptive) Property of *Moringa oleifera* Ethanol Leaf Extract in Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors INS and NOC designed the study and carried out all experiments. Author INS wrote the first draft of the manuscript. Author MGK performed the statistical analysis and managed literature. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: This study investigated the analgesic property of ethanol leaf extract of *Moringa oleifera* (ELMO) in rats.
Study Design: Different rat models (acetic acid, tail flick and tail immersion) were employed. The study was carried out in the Physiology Laboratory of the Department of Veterinary Physiology, Pharmacology, Biochemistry and Animal Health, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria, between July and December, 2013.
Methodology: 42 albino Rats were used for each experimental model. Varying doses of ELMO: 200, 300, 400 and 500 mg/kg were administered orally to rats in different groups. The negative control group received 0.2ml normal saline while Aspirin (10mg/kg) and Dicloferanc Potassium (2mg/kg) were used as reference drugs.
Results: ELMO exerted a dose dependent reduction in number of writhes per time in the acetic acid model as the varying doses used inhibited the number of writhes observed in the negative control group by 33.33, 57.84, 67.16 and 66.67% respectively. ELMO also

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increased reaction times in the tail flick and tail immersion models with 400mg/kg producing inhibition ratios of 10.05 and 11.51 respectively. Results in the treated groups were significantly (P<0.05) different from that of the negative control group and compared favorably with those of Aspirin and Diclofenac Potassium.
Conclusion: The experiments hence indicate that ELMO could contain principles with strong analgesic properties and seems promising in the search for new analgesic agents with minimal side effects and high potency for the relief of all forms of pain.

Keywords: Analgesic; aspirin; assay; cyclo-oxygenase-2; diclofenac potassium; Moringa oleifera; pain; prostaglandin.

1. INTRODUCTION

For decades, man had exploited wild plants for reasons of cost, availability and accessibility in his deliberate attempt to bring solution to his numerous health challenges. Today, the use of medicinal plants have become even more popular and is seen even by orthodox practitioners as the easiest means of drug(s) discovery and development. Available literature had reported that the primary aim of sourcing for plant drug through any of the known strategies and through the ethnomedical survey program is mainly, to detect the active (chemical) ingredients in plants, that exert some definite pharmacological effects in the body, since the results of such investigations would most often serve as a lead for the biological evaluation of these plants and to new drug discovery [1]. *Moringa oleifera* is only one of the numerous plants that are being exploited.

Moringa oleifera is a fast growing evergreen deciduous, perennial tree which grows to a height of 10-12m with trunk which may reach 45cm. The plant is slender with drooping and brittle branches. The leaves are feathery, pale green, compound tripinnate and 30 - 60 cm, with many small leaflets. Flowers are white or creamy with fragrant smell and are bisexual [2]. The plant, belonging to family *Moringaceae* and commonly known as horse radish tree or drum stick is reported to be used in Phytomedicine as antioxidant, antimicrobial, anti-inflammatory, antipyretic, antiulcer, anti-diabetic, anti-tumor and as a hypocholesteromic agent [3].

Analgesia is the absence of the sense of pain without loss of consciousness, while Analgesics are medicines that provide temporary relief from pain. These agents may act centrally by reducing the flow of pain signals from the brain and include opium, morphine, heroine, codeine [4] or may act locally at the actual site of pain to reduce the amount of pain causing chemicals produced e.g Ibuprofen, Diclofenac Sodium, Aspirin, and lots more.

The use of *Moringa oleifera* leaf extract in traditional medicine for the treatment of pyrexia and arthritic pains was the motivating factor for this present work which sought to investigate the analgesic effect of ELMO in rats with a view to validating this traditional claim.

2. MATERIALS AND METHODS

2.1 Collection of Plant and Preparation of Extract

Fresh leaves of *Moringa oleifera* were collected from a farm settlement in Umuakwela, Obodo Ahiara in Ahiazu Mbaise Local Government Area of Imo State, Nigeria. The extract

was prepared using the method of Akah et al. [5]. The collected leaves were air dried at room temperature for 7 days after which they were ground to coarse powder using a manual blender. The powdered material weighing 35g was introduced into the extraction chamber of the soxhlet extractor and extraction was done using ethanol as solvent. Extraction temperature was maintained at 78ºC for 48hours. At the end of the period, the ethanol was evaporated at low temperature in an electric oven to obtain a crude extract which weighed 10.50g and represented a yield of 30%.

2.2 Animals

A total of 126 Rats (120-160g) obtained from the Animal Production unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, was used for the study. These animals were fed with standard pelleted feed (Vital Feed, Nigeria), with water ad libitum, but starved for 12 hours prior to commencement of Experiment. All Animal experiments were conducted in compliance with NIH guidelines for care and use of Laboratory Animals (Pub. No. 85-23, Revised 1985), as expressed by Akah et al. [5]. The study was carried out in the Physiology Laboratory of the Department of physiology, Pharmacology, Biochemistry and Animal Health, Michael Okpara University of Agriculture, Umudike, Nigeria.

2.3 Experiments

2.3.1 Effect of ELMO on acetic acid induced writhing test

Forty two rats were divided into 7 groups of 6 rats each. Group 1 was given 0.2ml normal saline and served as the negative control. Groups 2 and 3 received Aspirin, 10mg/kg body wt. (BW) and Diclofenac Potassium (2.0mg/kg) respectively, while groups 4, 5, 6 and 7 were treated with 200, 300, 400 and 500mg/kg ELMO. All treatments were done by the oral route. After 30 minutes of treatment, each rat was given an intraperitoneal (I.P) injection of 0.6% acetic acid at a dose of 10ml/kg body weight. The number of writhes (stretching of hind limbs and bending of trunk) made by each rat was counted for 30 minutes. This method was described by Anaga et al. [6], but was however modified.

Percentage inhibition was evaluated using the expression:

$$\text{Percentage Inhibition} = \{\frac{(\text{Writhes in control}) - (\text{Writhes in test})}{(\text{Writhes in control})}\} \times 100$$

2.3.2 Tail flick assay

A modified method of Esam, [7], was employed. Forty two rats divided into 7 groups of 6 rats each. Group 1 received 0.2ml normal saline, group 2 and 3 received Aspirin (10mg/kg) and Diclofenac Potassium (2mg/kg) respectively, while groups 4, 5, 6 and 7 were treated with 200, 300, 400 and 500mg/kg ELMO. All treatments were administered orally. After 30 minutes of treatment, the lower halves of the tails of the animals were individually dipped into a beaker of cold water (0.1ºC). The time taken for tail withdrawal from the water was recorded as the reaction time.

2.3.3 Tail immersion test

A modified method of Esam, [7], was employed. Forty two rats divided into 7 groups of 6 rats each. Group 1 received 0.2ml normal saline. Groups 2 and 3 received Aspirin (10mg/kg) and Diclofenac Potassium (2mg/kg) respectively, while groups 4, 5, 6 and 7 were treated with 200, 300, 400 and 500mg/kg ELMO. After 30 minutes of treatment, the lower 5cm portion of each animal's tail immersed in a water bath maintained at 55ºC. The time taken for tail withdrawal from the water was recorded as the reaction time.

Inhibition time ratios were evaluated for the tail flick assay and tail immersion test using the expression:

$$\text{Inhibition Ratio} = \frac{\text{Reaction time in test}}{\text{Reaction time in control}}$$

2.4 Statistical Analysis

All data were expressed as mean ± Standard Error of Mean (SEM) and analyzed using student's t-test. P values less than 0.05 at 95% level of significance for tests versus control were adjudged significant.

3. RESULTS

3.1 Effect of ELMO Acetic Acid Induced Writhing in Rats

All doses of ELMO used in the experiments significantly (P<0.05) decreased the mean number of writhes when compared to negative control, with maximum effect (67.16% inhibition) achieved at 400mg/kg. The effect of the extract compared favorably with those of the standard drugs (Table 1).

Table 1. Effect of ELMO on acetic acid induced writhing in Rats

Group	Treatment	Number of writhes Per 30 Minutes ± SEM	% Inhibition
1.	0.2ml normal saline	40.80±0.85	
2.	10mg/kg, Aspirin	20. 60±0.52*	49.50
3.	2.0mg/kg Diclofenac potassium	3.00±0.28*	92.65
4.	200mg/kg ELMO	27.20±0.34*	33.33
5.	300mg/kg ELMO	17.20±0.34*	57.84
6.	400mg/kg ELMO	13.40±0.53*	67.16
7.	500mg/kg ELMO	13.60±0.60*	66.67

**P<.05 when compared to negative control group*

3.2 Effect of ELMO on Reaction on Time in Tail Flick and Tail Immersion Assays in Rats

All doses of the extract significantly (P<.05) increased the reaction times in tail flick and tail immersion assays. The effects were dose dependent and also compared favorably with those of standard drugs (Tables 2 and 3).

Table 2. Effect ELMO on reaction time in the tail flick assay

Group	Treatment	Reaction time in Minutes ± SEM	Inhibition Ratios
1.	0.2ml normal saline	0.84±0.04	1.00
2.	10mg/kg, Aspirin	3.64±0.08*	4.33
3.	2.0mg/kg Diclofenac Potassium	12.30±0.15*	14.64
4.	200mg/kg ELMO	5.69±0.09*	6.77
5.	300mg/kg ELMO	7.51±0.12*	8.94
6.	400mg/kg ELMO	8.44±0.07*	10.05
7.	500mg/kg ELMO	8.20±0.13*	9.76

**P<.05 when compared to negative control group*

Table 3. Effect of ELMO on reaction time in the tail immersion assay

Group	Treatment	Reaction time in Minutes ± SEM	Inhibition Ratios
1.	0.2ml normal saline	0.75±0.03	1.00
2.	10mg/kg, Aspirin	2.75±0.10*	3.67
3.	2.0mg/kg Diclofenac Potassium	11.50±0.08*	15.33
4.	200mg/kg ELMO	7.38±0.09*	9.84
5.	300mg/kg ELMO	7.77±0.10*	10.36
6.	400mg/kg ELMO	8.63±0.16*	11.51
7.	500mg/kg ELMO	9.15±0.18*	12.20

P<.05 when compared to negative control group

4. DISCUSSION

The ethanol extract of *Moringa oleifera* leaves reduced the number of writhes and increased reaction time in rats exposed to pain stimuli via the different models - acetic acid induced writhing, the tail flick assay and the tail immersion assay. These results suggest that the extract contain active principles with analgesic properties. The phytochemical components of *Moringa oleifera* leaf extract had been reported to include alkaloids, glycosides, phenols, saponins and tannins [8], some of which may be implicated in the induction of analgesia. The extract may have achieved this analgesic effect by inhibiting the activity of cyclo-oxygenase-2 (cox-2) which results to the stopping of prostaglandins formation. Prostaglandins are chemical substances which stimulate the noci receptors in the body leading to the sensation of pain. The extract may also have interfered with G-protein mediated signal transduction, an analgesic mechanism unrelated to inhibition of prostaglandin synthesis. It also may have augmented the peripheral mechanism through interference with the formation of prostaglandins in the central nervous system. These mechanisms have been implicated in the forms of analgesia induced by non-steroidal anti-inflammatory drugs (NSAIDs), including Aspirin and Diclofenac Potassium [9,10,11]. The result of this study tends to agree with Suleiman [12], who reported that *Moringa oleifera* aqueous extract possess anti-nociceptive and anti-inflammatory activities and corroborates with the use of *Moringa oleifera* leaves in traditional medicine for the relief of pain and inflammations [3,13,14].

5. CONCLUSION

These results suggest that *Moringa oleifera* leaf extract is a safe and potent agent capable of relieving pains and may be of benefit in the management of all forms of Arthritis, Rheumatism and other conditions associated with pain. With the gastrointestinal toxicity associated with the use of Aspirin and Diclofenac Potassium, *Moringa oleifera* leaf extract could be used as a main therapy for the relief of pains.

ETHICAL APPROVAL

The Authors declare that this work was not against public interest. Animal experiments were conducted in accordance with NIH guidelines for care and use of Laboratory animals (Pub. No. 85-23, Revised 1985).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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