OPEN ACCESS
Scholars International Journal of Biochemistry
Abbreviated Key Title: Sch Int J Biochem
ISSN 2616-8650 (Print) |ISSN 2617-3476 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

# Anti-Nociceptive Properties of Methanolic Extract of *Artocarpus altilis* (Breadfruit) on Wistar Rats in Mechanical Model of Pain Study

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**DOI:** 10.36348/sijb.2023.v06i04.001 | **Received:** 24.02.2023 | **Accepted:** 10.04.2023 | **Published:** 15.04.2023

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## Abstract

Flavonoids has been shown to have anti-inflammatory and anti-oxidant properties, which may have potential protective effects against mechanical induced models of pain. In this context, administering methanolic extract of *artocarpus altilis* (MEAA) during the experimental rats could potentially increase the pain threshold, which could in turn, increase the quality of health and life expectancy. Dearth literature exists on the analgesic effects of Bread fruit on pain. Hence, this study aims at investigating the potential anti-nociceptive properties of MEAA to increase paw withdrawal latency using murine model. 25 *Wistar* rats were randomly selected into five groups: Group 1=(control); Group 2, 3 & 4 (100, 200, 300-mg/kg MEAA, respectively) and Group 5=100-mg/kg Aspirin (ASP). The rats were subjected to mechanical pain-behavioral studies at 30, 60, 10 and 120 minutes after administration of test substances. Results obtained showed that, at 30 minutes post administration of MEAA and ASP, significant increase ($p<0.05$) in pain threshold was observed in 100, 200, 300-mg/kg and ASP groups when compared to control. At 60 minutes, significant increase ($p<0.05$) in pain threshold was observed in 200, 300-mg/kg and ASP groups when compared to control. Finally, at 90- and 120-minutes, significant increase ($p<0.05$) in pain threshold was observed in 300-mg/kg group only, when compared to control. In conclusion, graded doses of MEAA showed analgesic properties by increasing pain threshold when administered 30, 60, 90 and 120 minutes prior to subjection of the animals to mechanical pain. Therefore, MEAA have shown analgesic properties that could even be more effective than the reference drug (aspirin).

**Keywords:** MEAA, Pain, Anti-nociceptive, Analgesic, Brain, Mechanical model of pain.



## 1.0 INTRODUCTION

### 1.1 Background

Pain is an uncomfortable sensation that is frequently brought on by strong or destructive stimuli, such as stubbed toes, burned fingers, or applying alcohol to cuts [1]. According to the commonly accepted definition of pain provided by the International Association for the Study of Pain; it is "an unpleasant sensory and emotional experience related to actual or potential tissue damage or described in terms of such damage" [2]. To avoid repeat occurrences of the same thing, safeguard a wounded bodily part while it heals, and withdraw from harmful conditions are all motivated by pain [3]. Once the painful stimulus is eliminated and the body has healed, the majority of discomfort quickly goes away [4, 5]. When a stimulus has been removed and the body appears to be recovering, pain might nevertheless last. Other times, pain can develop even in the absence of any visible injury, sickness, or stimulus [6].

In both developed and developing nations, the most frequent cause for consulting a doctor is pain [7]. It is a common major symptom of many illnesses and can seriously impair a person's functioning in daily life [8]. Social support, hypnotic suggestion, enthusiasm, or diversion are examples of psychological factors that can greatly alter the intensity or unpleasantness of pain [9, 10]. In response to strong stimuli, nociceptors produce trains of action potentials [11]. The severity of the pain is influenced by the firing frequency [12]. Located in the skin are nerve endings known as nociceptors that are capable of detecting potentially harmful mechanical, thermal, and chemical stimuli [13, 14]. Additional internal surfaces where they can be found include the periosteum, joint surfaces, and some internal organs

[15]. The body's nociceptors are concentrated in different places; the skin contains more of them than deep interior surfaces do [16]. In the dorsal root ganglia, which is outside the spinal column, are the unspecialized free nerve endings known as nociceptors [17]. The axons that connect the receptors to the spinal cord or brain are used to categorize different types of nociceptors [14[. Nociceptors have a threshold, meaning that they need a particular amount of stimulation before sending a signal [14].

A signal is transmitted into the spinal cord along the neuron's axon once this threshold is met [18]. In order to research pain, nociceptive threshold testing purposefully subjects either humans or animals to a noxious stimuli [18]. The method is frequently applied to animals to investigate the effectiveness of analgesics and to determine dosage levels and duration of action [19]. The substance being tested is administered after establishing a baseline, and the elevation in threshold is noted at predetermined time intervals [19]. The threshold ought to go back to its initial (pre-treatment) value once the medicine wears off [20].

To control nociception and pain, the body has an endogenous analgesia system that can be reinforced with analgesic medications [21]. The degree to which nociception reaches the higher brain areas is reduced by both a central nervous system analgesia mechanism and peripheral receptors [14]. Before pain reaches the thalamus and consciousness, it can be altered by the periaqueductal gray [22]. In accordance with the gate control theory of pain, this region can also lessen pain when nociception is combined with non-painful stimuli (Ropero and Taniguchi, 2016). Some drugs have been known to modulate and mitigate the effects of pain.

Opiates and nonsteroidal anti-inflammatory medications (NSAIDs) are frequently utilized in these situations [24]. However, after taking these medications, users have reported experiencing side effects such dependency, renal damage, respiratory depression, gastrointestinal disturbances, and renal damage [24]. This has caused researchers to consider using natural plants and ayurvedic medicine to create novel anti-inflammatory and analgesic medications that may also have fewer negative effects [25]. Such natural plant as bread fruit, which has been reported of flavonoid constituents [26, 27], is a phytochemical of choice.

An *Artocarpus altilis* species of flowering tree belongs to the Moraceae family of mulberries. Greek (artos = bread, karpus = fruit, and altilis = fat) is the source of the scientific or Latin name. Its name is derived from the texture of the cooked medium mature fruit, which has a potato-like flavour, akin to freshly baked bread. it grows across Southeast Asia, South India and most Pacific Ocean islands. It is also grown in the Leeward Islands and islands of the Carribean and in Africa. Breadfruit contains flavonoids found in various other plants.

Flavonoids has been shown to have anti-inflammatory and anti-oxidant properties, which may have potential protective effects against mechanical induced models of pain [28, 29]. In this context, administering MEAA during the experimental rats could potentially increase the pain threshold, which could in turn, increase the quality of health and life expectancy [8]. Investigating the potential anti-nociceptive properties of MEAA to increase paw withdrawal latency using murine model is important due to the growing prevalence low life expectancy as a result of damages to major tissues and organs due to pains sensation thereby serving as analgesic agents.

## 2.1 MATERIALS AND METHODS

### 2.1.1 Drugs and chemicals

Aspirin tablet was obtained from the University of Port Harcourt Teaching Hospital Pharmacy Department and was used as the reference anti- ulcer drug. Aspirin tablet weighing 100 mg was dissolved in 10ml of distilled water to make a stock solution of 2 mg/mL to be administered at a dose of 10 mg/kg per oral (po).

**Plant material and preparation of extract**

*Artocarpus altilis* (breadfruit) were purchased from local food vendors in Port Harcourt. The fruits were identified and confirmed for use by a botanist of the department of plant science biotechnology (PSB) herbarium, University of Port Harcourt, Nigeria. Afterwards, sample specimens were deposited in the herbarium of the department for future references. The *Artocarpus altilis* samples were dried under room temperature, after which, were weighed and grinded into powder form. Powdered dried *Artocarpus altilis* (7.5kg) was extracted by cold extraction for 72 hours using methanol (meOH). The meOH extract provided a semi-solid residue (MEAA: 7.2kg) (Odec, 2001), and the percentage yield is 96 percent. The extract was evaporated to semi-solid form and stored in the refrigerator throughout the period of the experiment to preserve the prepared extract.

### 2.1.2 Experimental Animals and housing

25 Wistar rats of comparable sizes of 160 – 180g were bought from the Animal House of the Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria. The animals were housed in a forced air facility before and during the experiment at the Animal House in the University of Port Harcourt. The animals were maintained in the cages at 23°C and 50 – 60% relative humidity, with maximum ventilation. The groups were kept in separate compartments of different plastic cages measuring 23.5 x 16.5 x 12cm (length, height, and width respectively) and were always given water and feeds *ad libitum*. The

experiment described in this report was conducted in accordance with the National Institute of Health (2002) guide for the care and use of laboratory animals. These guides were approved by the institutional ethics committee for animal experiment.

### 2.1.3 Acclimatization of Animals

The rats used for the study were acclimatized for two weeks at the animal house to observe them before the commencement of the experiment under standard laboratory condition in a well-ventilated standard housing condition.

### 2.1.4 Ethical statements

The experimental procedures and techniques used in the study were in accordance with acceptable principles for laboratory animal use and care by NIH, 1985 and EU directive of 1989:86/609/EEC. All conditions and handling of the animals were approved by the Ethical Committee of Faculty Basic Medical Sciences, University of Port Harcourt, Nigeria.

### 2.2 Experimental Design and animal grouping

After the two weeks of acclimatization, the twenty-five (25) rats were randomly divided into five groups of five rats (3 male and 2 females) each. The control and reference groups received 10 ml distilled water and 100mg/kg of aspirin; while the three test groups received 100 mg/kg, 200 mg/kg and 300 mg/kg of *Artocarpus altilis* respectively. Solutions of *Artocarpus altilis* at the above dosage were administered orally to 12 hours fasted Wistar rats according to their body weights. Thirty minutes after administration, sensory thresholds were measured with an anagelsimeter (NatureGene Corp., Medford, NJ). This device is based on Randall-Selitto (Randal and Selitto, 1957), and was used to apply a linearly increasing pressure by means of a blunt Perspex cone, to the palmer region of the right forepaw of the Wister rat until the rat withdraws the paw, the value was recorded as the threshold for mechanical pain. This process was repeated after 60 minutes, 90 minutes, and 120 minutes after administration. The response of *Artocarpus altilis* and aspirin treated groups were recorded and compared with those animals in the control group (distilled water, 10 mL/kg). A summary of the experimental design is presented below:

- GROUP A-10 mg/mL of Distilled water (control).
- GROUP B-100mg/kg of Methanolic extract of A*rtocarpus altilis (MEAA).*
- GROUP C-200mg/kg of Methanolic extract of *Artocarpus altilis (MEAA).*
- GROUP D-300mg/kg of Methanolic extract of *Artocarpus altilis (MAA).*
- GROUP E-Standard drug 100 mg/kg of aspirin.

## 3.0 STATISTICAL ANALYSIS OF DATA

Statistical data were analysed using GraphPad Prism 8 software (Graph-pad Software Inc., San Diego, USA). Multiple-group parametric data were analysed by one-way analysis of variance (ANOVA), expressed as mean standard error of mean (SEM); followed by a Tukey's *post hoc* test for multiple group comparisons. Data was considered statistically significant when $p \leq 0.05$.

## 4.0 RESULTS

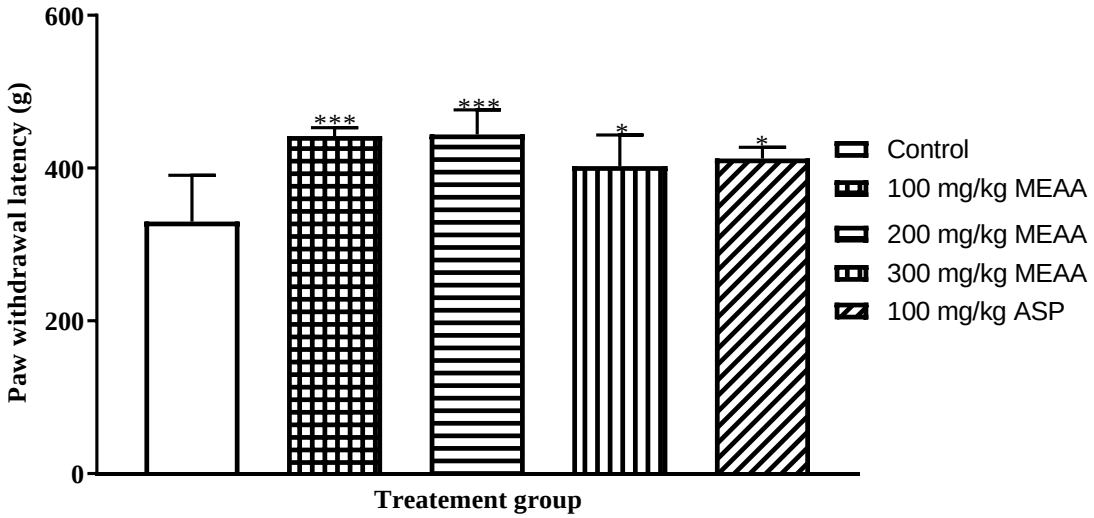


**Fig. 1: Anti-nociceptive properties of methanolic extract of *Artocarpus altilis* (breadfruit) on Wistar rats in mechanical model of pain study (30 minutes after administration of test drugs).**

Data presented as mean ± standard error of mean. *Significantly different at $p < 0.05$; **MEAA** = methanolic extract of *Artocarpus altilis*; **ASP** = Aspirin.

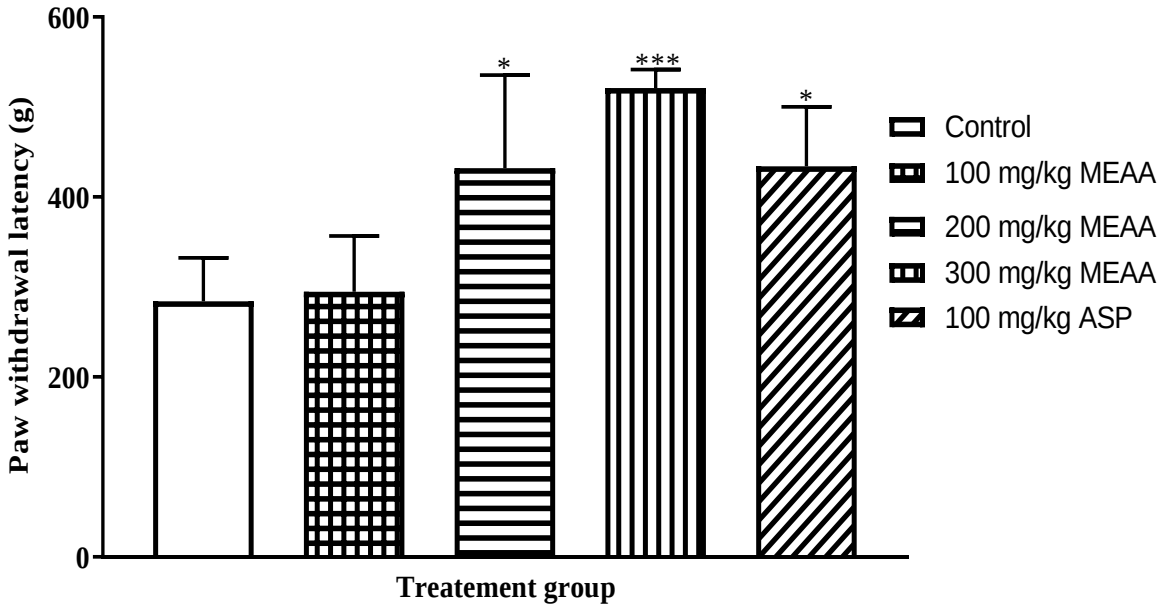


**Fig. 2: Anti-nociceptive properties of methanolic extract of *Artocarpus altilis* (breadfruit) on Wistar rats in mechanical model of pain study (60 minutes after administration of test drugs).**

Data presented as mean ± standard error of mean. *Significantly different at $p < 0.05$; **MEAA** = methanolic extract of *Artocarpus altilis*; **ASP** = Aspirin.

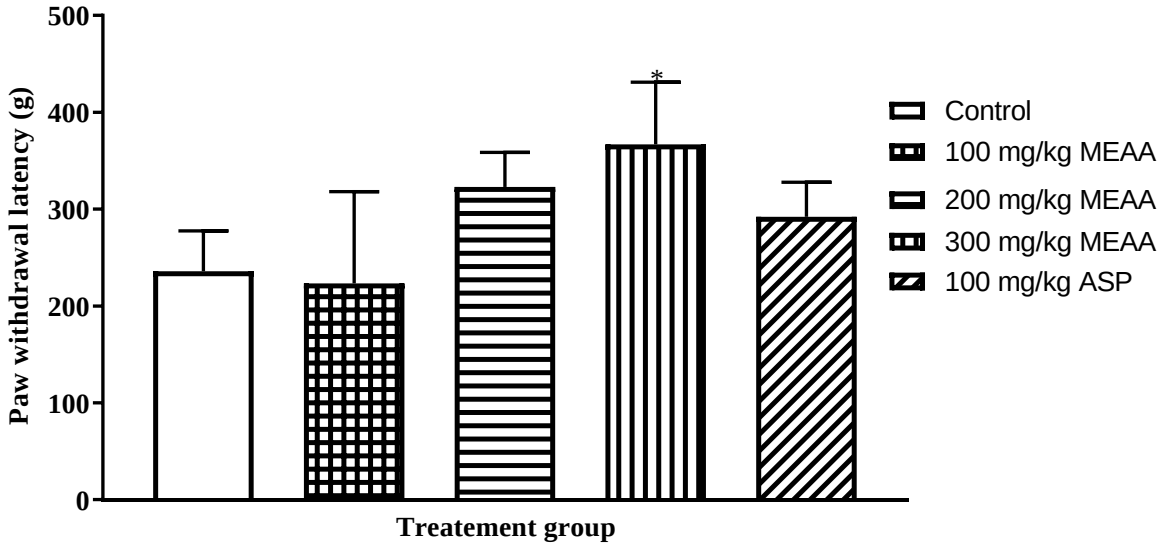


**Fig. 3: Anti-nociceptive properties of methanolic extract of *Artocarpus altilis* (breadfruit) on Wistar rats in mechanical model of pain study (90 minutes after administration of test drugs).**

Data presented as mean ± standard error of mean. *Significantly different at $p < 0.05$; **MEAA** = methanolic extract of *Artocarpus altilis*; **ASP** = Aspirin.

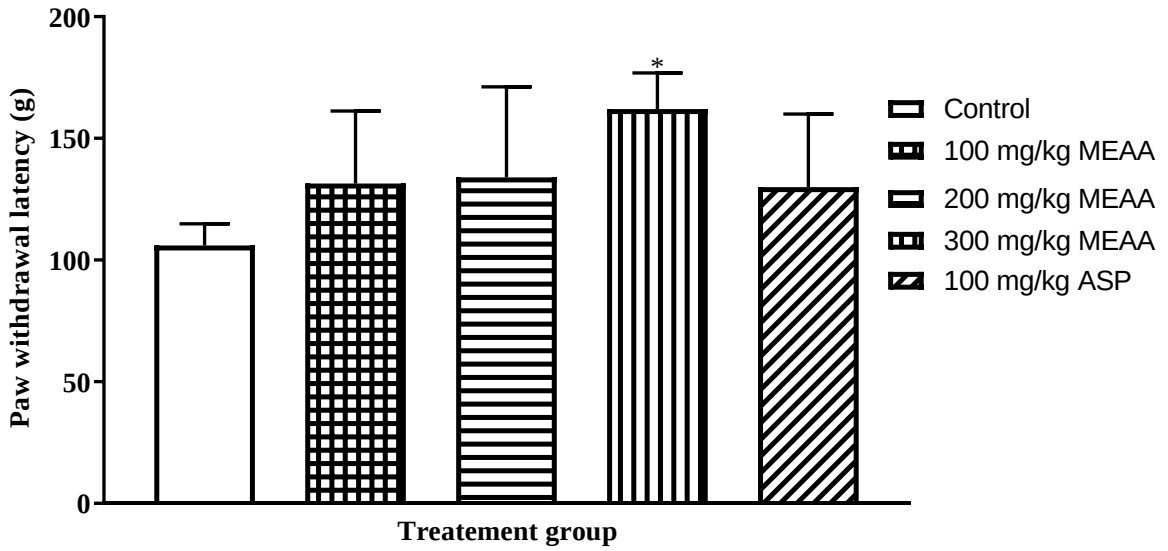


**Fig. 4: Anti-nociceptive properties of methanolic extract of *Artocarpus altilis* (breadfruit) on Wistar rats in mechanical model of pain study (120 minutes after administration of test drugs).**
Data presented as mean ± standard error of mean. *Significantly different at $p < 0.05$; **MEAA** = methanolic extract of *Artocarpus altilis*; **ASP** = Aspirin.

## 5.1 DISCUSSION

This study was carried out to determine the effect of *Artocarpus altilis* on pain tolerance in Wistar rats using the Randall–Selitto paw pressure test to evaluate the withdrawal latency over a period of time. The results reveal varying values of pain tolerance recorded within the different groups, a statistically significant difference ($p < 0.05$) of pain tolerance was recorded in the pain tolerance of rats.

The results obtained from this study shows that elevated pain threshold was observed in groups B, C, D and E after 30 minutes administration of MEAA (100, 200, 300 mg/kg) and ASP (100 mg/kg) when compared to control (Figure 1). Similarly, the outcome of the study shows that elevated pain threshold was observed in groups C, D and E after 60 minutes administration of MEAA (100, 200, 300 mg/kg) and ASP (100 mg/kg) when compared to control (Figure 2); with the 300 mg/kg MEAA showing more pain tolerance than the rest of the groups, including the reference drug (100 mg/kg ASP). Additionally, recorded results shows that elevated pain threshold was observed only in groups D after 90 minutes of MEAA (100, 200, 300 mg/kg) and ASP (100 mg/kg) administrations when compared to control; this means that 300 mg/kg MEAA shows more pain tolerance than the rest of the groups, including the reference drug (100 mg/kg ASP) (Figure 3). Finally, figure 4 shows that elevated pain threshold was observed in groups C, D and E after 120 minutes of MEAA (100, 200, 300 mg/kg) and ASP (100 mg/kg) administrations when compared to control; with the 300 mg/kg MEAA showing more pain tolerance than the rest of the groups, including the reference drug (100 mg/kg ASP).

This study validates Karthik *et al.*, (2022), who investigated the analgesic and anti-inflammatory prospective of ethanolic leaf extract of *Artocarpus hirsutus Lam in Wistar rats*. He reported that oral administration of graded doses of 100, 200, and 400 mg/kg body weight to rats, exhibited analgesic effects and anti-inflammatory effects. Though the current work did not report the anti-inflammatory effect of MEAA, but a significant analgesic activity of a related plant *Artocarpus Artocarpus hirsutus.* Although, [31] reported that *Artocarpus altilis* leaf and bark extracts are safe to be utilized as functional ingredient in pharmaceutical agents or as nutraceutical.

The results revealed varying analgesic effects of the *Artocarpus altilis* extract on pain tolerance with doses ranging from 200-300mg/kg showing similar analgesic properties to the reference drug (Aspirin), with a very high pain tolerance after 60 minutes of the extract and reference drug administration in the rats, this is in agreement with the findings of [32] which reported similar analgesic effects of leave extract of *Artocarpus altilis* extract in acetic acid induced pain model of mice compared to mefenamic acid. This study shows that the analgesic effect of MEAA is dose dependent with the high dose (300 mg/kg) showing more analgesic activity overtime than the rest of the groups. Although the 100 mg/kg MEAA showed early analgesic activities more than the rest of the groups, the effects of that action wear off more rapidly as it did not show any impact on the long run (90 and 120 minutes [20]. This test can distinguish the central and peripheral action by the comparison of pain caused by the compression of inflamed paw or non-inflamed paw

[33]. The pain caused by the compression of non-inflamed hind paw of rats is centrally mediated and are attributed to the direct stimulation of nociceptor afferent fibres [34]. Therefore, we suggest that MEAA may be elucidating its action through central antinociceptive mediators. It is worthy to note that the anti-nociceptive activities of MEAA as shown by an increase in pain threshold through paw withdrawal was more effective than that of the standard reference drugs (100 mg/kg) aspirin.

## 6.0 CONCLUSION

In conclusion, graded doses of MEAA showed analgesic properties by increasing pain threshold when administered 30, 60, 90 and 120 minutes prior to subjection of the animals to mechanical pain. Therefore, MEAA have shown analgesic properties that could even be more effective than the reference drug (aspirin) according to the results obtained from this present study.

Findings from this study suggest that the administration of MEAA to adult Wistar rats can play a potential anti- nociceptive role against mechanical induced pain damage. However, studies in humans should be considered and if there are positive outcomes, then breadfruit should be considered as a potential target for intervention against pain. This will have a direct impact on the global health care systems as it will aid in the improvement of quality of life and general functioning. Also, the mechanisms of which MEAA exerts its anti-nociceptive role require further investigation. Finally, the various flavonoids constituents of breadfruits should be explored and characterized to evaluate the flavonoid responsible for the analgesic action of MEAA.

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