Cardoso-Daodu *et al* Pan African Journal of Life Sciences (2023): 7(3): 723-733
DOI: 10.36108/pajols/3202/70.0350

Publication of Faculty of Basic Medical Sciences and Basic Clinical Sciences , Ladoke Akintola University of Technology, Ogbomoso

www.pajols.org
Online ISSN:2672-5924

RESEARCH **Open Access**

Evaluation of Anti-nociceptive and Anti-Inflammatory Effects of *Markhamia tomentosa* Cream Co-Formulated with *Eucalyptus* Oil

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Received 9th October 2023; Revised 30th October 2023; Accepted 6th November 2023

ABSTRACT

Background: Pain is a noxious, sensory, and emotional experience due to intense stimuli. Pain is intimately linked with nociception. The clinical therapies available at the moment for pain are tricyclic antidepressants, non-steroidal anti-inflammatory drugs, and opioids. There is a need to develop and investigate safer alternatives that minimise the chances of psychological or physical drug dependence and drug dose tolerance. The purpose of this research is to develop an anti-inflammatory and anti-nociceptive formulation (*Markhamia tomentosa* cream co-formulated with *Eucalyptus* oil as a permeation enhancer) and evaluate its efficacy.

Methods: In this study, *Markhamia tomentosa* was extracted from its leaves using maceration technique. A qualitative screening of the *Markhamia tomentosa* ethanolic extract (EEMT) was carried out. Five emulsions (A1-A5) containing varying compositions of Markhamia tomentosa were developed, and a negative control (an emulsion that did not contain *Markhamia tomentosa* or *Eucalyptus* oil) was also developed using fusion method. The anti-inflammatory and anti-nociceptive properties of the topical creams were evaluated using mice paw-licking and xylene-induced ear oedema tests. Diclofenac and aspirin administered at 20 mg/kg and 50mg/kg orally, respectively, were used as standards. All experiments were carried out in triplicates.

Results: The phytochemical investigation of the *Markhamia tomentosa* extract showed a marked presence of key phyto-constituents such as saponins, flavonoids, alkaloids, and terpenoids. A1 and A2 had significantly higher percentage of nociception inhibition at phase I—neurogenic and phase II—inflammatory stages compared to Diclofenac 20 mg/kg. A1 and A3 had anti-inflammatory properties more potent than Aspirin 50mg/kg. In both tests, the negative controls did not show any level of potency. All results in this investigation were expressed as mean ± S.D (n=3) and ($p<0.05$) for all data sets. Formulation A1 had the highest concentration of EEMT. No rash, erythema, or oedema was observed in the Draize test implying that the formulations are safe for topical application.

Conclusion: *Markhamia tomentosa* and eucalyptus oil cream showed potent anti-nociceptive and anti-inflammatory activity. *Markhamia tomentosa* cream co-formulated with *Eucalyptus* oil as a permeation enhancer can be used effectively as a non-opioid, anti-nociceptive, and anti-inflammatory cream topically for pain and its symptoms.

Keywords: Pain, Nociceptors, Inflammation, *Markhamia tomentosa*, Paw-licking

1.0 INTRODUCTION

Pain is a bad, sensory, and emotional experience due to intense chemical, heat, or mechanical stimuli [1]. These stimuli are identified by receptors known as nociceptors. Pain and nociception are closely linked. However, nociception can best be described as the neurological processing and encoding of intense and noxious stimuli in the human body. Nociceptive signals are mainly expressed as a pain sensation [2]. Pain is a feeling sometimes characterized by inflammation. The cardinal signs of inflammation are oedema, erythema, heat and pain. Anyone can suffer from pain as a result of illness, trauma, or surgery. Inflammation, as mentioned earlier, is a hallmark symptom that accompanies pain, and it occurs when leukocytes migrate to damaged or injured tissues to protect the body from harm [3]. Inflammation can be divided into three stages: the silent, vesicular, and cellular stages [4]. Severe inflammation may predispose an individual to ailments such as cardiovascular diseases, intestinal bowel disease, colorectal cancer, and neurodegenerative diseases. It is, therefore, imperative that pain alongside its symptoms be promptly and adequately treated [5].

Tricyclic antidepressants, non-steroidal anti-inflammatory drugs, and opioids (in no particular order) are well-established drugs for pain [4]. Unfortunately, continuous use of these drugs may lead to terrible side effects such as psychological and physical drug dependence, drug dose tolerance, cardiovascular diseases, and gastrointestinal dysfunction [6]. As a result, there is a burgeoning interest in the utilization of plant-sourced medicinal materials for pain treatment, as they are less likely to cause drug dose tolerance, drug dose dependence, or severe side effects experienced through pain therapy [7].

Markhamia tomentosa is a plant from the family Bignoniaceae. It is a tree about 5-30 feet high and has large yellow flowers. It is indigenous to tropical regions such as Nigeria, Congo, and other parts of Africa and Asia [8]. *M. tomentosa* is popular for its anticancer properties [9]. *M. tomentosa* is composed of phyto-constituents such as oleanolic acid, pomolic acid, flavonoids, tormentic acid, phenols, β-sitosterol, steroids, saponins, terpenes, tannins and alkaloids [10]. Traditionally the root, barks and leaves of *M. tomentosa* are used to treat several diseases, such as gout, scrotal elephantiasis, diarrhoea, rheumatoid arthritis, and cancer. Its anti-nociceptive and anti-inflammatory properties are a relatively novel concept [10].

Eucalyptus globulus belongs to the family Myrtaceae and the sub-class Rosidae [11]. It is found in the tropical and subtropical regions of Africa, Asia and America. The eucalyptus is widely cultivated in plantations in Portugal, South Africa, Spain, Brazil, India, and China. The eucalyptus plant has inevitably caught the interest of scientists as it is the plant source of *Eucalyptus* oil, which has numerous significant health benefits [12]. It is used traditionally for muscle aches and pain as an antitussive, anti-inflammatory, antioxidant and antimicrobial agent. The main active ingredient of eucalyptus oil is eucalyptol (1, 8-cineole), which constitutes up to 70% of the oil [13]. Eucalyptol has well-established and potent anti-inflammatory and anti-nociceptive properties [14].

There is a need to investigate safer alternatives that minimize the chances of psychological or physical drug dependence and drug dose tolerance. In this study, *M. tomentosa* topical cream with *Eucalyptus* oil as a permeation enhancer was formulated, and the anti-nociceptive and anti-inflammatory effects of the formulation were investigated [15]. The study uses the synergistic interaction between *M. tomentosa* and *Eucalyptus* oil to produce a potent anti-inflammatory and anti-nociceptive topical cream [16]. Presently and to the best of our knowledge, no *M. tomentosa* and *Eucalyptus* oil cream has been formulated to investigate its anti-inflammatory or anti-nociceptive properties. Herein lies the novelty of this study. The fact that the formulation is a topical cream serves as an advantage because this eliminates the chances of psychological or physical drug dependence and drug dose tolerance that often results from pain therapy [17, 18].

2.0 METHODOLOGY

2.1 Materials

Materials used for this study include Eucalyptus oil (Spectrum Chemicals, California, USA), Carbopol Ultrez (Sigma Aldrich, St. Louis USA), Amaranth dye (Sigma Aldrich, St. Louis USA), stearic acid (Surfachem, UK), cetostearyl alcohol (Pure Nature, NZ), soft paraffin (Unicorn petroleum, India), hard paraffin (Okchem, China), liquid paraffin (Okchem, China), triethanolamine (Merck, Germany), methyl paraben and propyl paraben (Sigma Aldrich, St. Louis USA).

2.1.1 Plant Collection and Authentication

The leaves of *M. tomentosa* were obtained from Oke-Igbo, Ondo state, Nigeria, in June 2019. The taxonomic identification and authentication of the plant were carried out in the Herbarium of the Department of Botany and

Microbiology, University of Lagos. A herbarium specimen was deposited with voucher number LUH 8056 [19].

2.1.2 Extraction of the Plant Material

The leaves of *M. tomentosa* were washed and dried in an oven at 50℃. The dried leaves were pulverized to a coarse powder using a laboratory grinding mill. Then, 700g of the coarsely powdered leaves were macerated in ethanol for three days. The extract was filtered using a muslin bag. The extract was concentrated in the oven at 50℃ to obtain *M. tomentosa* ethanolic leaf extract (EEMT) [19].

2.2 Phytochemical Screening

2.2.1 Test for terpenoids (Libermann Burchard test)

Firstly, 1g of Markhamia tomentosa leaf powder was dissolved in chloroform and filtered. Acetic anhydride was added to the filtrate and mixed thoroughly. Two drops of concentrated sulphuric acid were added to the filtrate to give a greenish colour, which concluded the presence of terpenoids [20].

2.2.2 Test for anthraquinone glycosides

Exactly 0.5g of *M. tomentosa* leaf powder was boiled with $FeCl_3$ and dilute HCl, then filtered hot. Chloroform (2mls) was added to the filtrate and shaken well. The organic phase was separated from the two-phase mixture with a pipette. Dilute ammonia (1ml) was added to the organic phase slowly. The occurrence of a pink or red colour indicated the presence of anthraquinone glycosides [21].

2.2.3 Test for cardiac glycosides (Keller-Killiani test)

M. tomentosa leaf powder (0.5g) was measured and transferred into a test tube; three drops of $FeCl_3$ and 1 ml of glacial acetic acid were added. The mixture was then filtered into a clean test tube containing concentrated sulphuric acid. The appearance of a reddish-brown ring at the interface between the two layers and a bluish-green upper layer concluded the presence of cardiac glycosides [22].

2.2.4 Test for Phenolic compounds

M. tomentosa leaf powder (1g) was dissolved in chloroform and filtered. To the precipitate, three drops of lead acetate were added. The appearance of a white precipitate proved the presence of phenols [23].

2.2.5 Test for alkaloids (Mayer's test)

Exactly 0.5g of *M. tomentosa* leaf powder was dissolved in 5 ml of water and then filtered. A few drops of the filtrate were placed on a white tile, and two drops of Mayer's reagent were added. The appearance of a white precipitate indicated the presence of alkaloids [21].

2.2.6 Test for saponins

Exactly 0.5g of *M. tomentosa* leaf powder was dissolved in 5 ml of water by heating and then filtered. The filtrate was shaken vigorously, and the appearance of a persistent froth confirmed the presence of saponins [23].

2.2.7 Test for tannins (Ferric chloride test)

Firstly, 0.5g of *M. tomentosa* leaf powder was dissolved in 5 ml of water and then filtered. Two drops of ferric chloride were added to the filtrate, and a blue-black colour confirmed the presence of tannins [21].

2.2.8 Test for flavonoids (Alkaline reagent test)

Exactly 1g of *M. tomentosa* leaf powder was dissolved in 5 ml of water and then filtered. Ammonium hydroxide solution (10%) was added to the filtrate. A yellow fluorescence concluded the presence of flavonoids [20].

2.3 Formulation Development

The fusion method was used to prepare a water-in-oil emulsion. The surfactant (stearic acid), oil phase (cetyl stearyl alcohol, soft, liquid, and hard paraffin), and the aqueous phase (methyl paraben, propyl paraben, triethanolamine, water and ethanol extracts of *M. tomentosa* leaves) were individually heated in a water bath to 80°C. The emulsifier stearic acid and the aqueous phase were gradually added into the oil phase with constant stirring. Carbopol Ultrez was dissolved in water and utilised as a thickener by addition to the cream base. The *Eucalyptus* oil was then added after the temperature dropped to (45 ± 0.5)℃ (Table 1) [24].

2.4 Determination of the Emulsion Type

To ascertain the emulsion type of formulations A1-A5 and control, three drops of Amaranth dye were added to each formulation (0.4g). All formulations were examined for the phase type using a microscope (Eclipse E100-LED Upright Microscope, Nikon NY, USA) [25].

2.5 Microscopic Examination

Microscopic examination was carried out for each cream (A1-A5 and Control) by preparing a smear of each formulation on separate slides and covering them with coverslips. The slides were then viewed under the microscope (Eclipse E100-LED Upright Microscope, Nikon NY, USA) [26].

Table 1. Formulas for the Preparation of Formulations A1-A5 and the Control Formulation

Ingredients	A1	A2	A3	A4	A5	Control
EEMT(mg)	1 x10^{3}	300	250	200	150	-
EO (L)	5 x 10^{-4}	5 x 10^{-4}	5 x 10^{-4}	5 x 10^{-4}	5 x 10^{-4}	-
Stearic acid (mg)	2.5 x 10^{3}	2.5 x 10^{3}	5 x 10^{3}	5 x 10^{3}	2.5 x 10^{3}	2.5 x 10^{3}
Cetostearyl alcohol (L)	3.75 x 10^{-3}	3.75 x 10^{-3}	5 x 10^{-3}	5 x 10^{-3}	5 x 10^{-3}	3.75 x 10^{-3}
Liquid Paraffin (L)	4 x 10^{-3}	4 x 10^{-3}	4 x 10^{-3}	4 x 10^{-3}	4 x 10^{-3}	4 x 10^{-3}
Hard Paraffin (mg)	2.5 x 10^{3}	2.5 x 10^{3}	2.5 x 10^{3}	2.5 x 10^{3}	2.5 x 10^{3}	2.5 x 10^{3}
Soft Paraffin (mg)	5 x 10^{3}	5 x 10^{3}	5 x 10^{3}	5 x 10^{3}	5 x 10^{3}	5 x 10^{3}
Methyl Paraben(L)	5 x 10^{-5}	5 x10^{-5}	5 x10^{-5}	5 x10^{-5}	5 x10^{-5}	5 x10^{-5}
Propyl Paraben(L)	2.5 x 10^{-5}	2.5 x10^{-5}	2.5 x10^{-5}	2.5 x10 $^{-5}$	2.5 x10 $^{-5}$	2.5 x10 $^{-5}$
Triethanolamine (L)	1 x 10^{-3}	1 x 10^{-3}	1 x 10^{-3}	1 x 10^{-3}	1 x 10^{-3}	1 x 10^{-3}
Carbopol Ultrez (mg)	5 x 10^{2}	1 x10^{3}	1.5 x 10^{3}	5 x 10^{2}	1 x10^{3}	5 x 10^{2}
Purified water (L) to	1 x 10^{-1}	1 x 10^{-1}	1 x 10^{-1}	1 x 10^{-1}	1 x 10^{-1}	1 x 10^{-1}

2.6 pH, Rheological evaluation, Organoleptic test and Spreadability and Skin Irritancy Test (Draize Test)

pH; the pH of the formulations (A1-A5 and control) was obtained using a pH meter. The electrode was immersed in each cream for 50 sec to allow for equilibration. Measurements were performed in triplicates [24].

Rheological evaluation; the viscosity of the creams (A1-A5 and control) was determined at 24 °C at 10–50 rpm using a Spindle 5.0, cone and plate viscometer (DV-E Digital viscometer, Brookfield Engineering Laboratories, Middleboro, USA) [27].

Organoleptic test; formulations (A1-A5 and control) were assessed for homogeneity by evaluating their visual appearance and texture [26].

Spreadability; the spreadability was given as time (seconds) taken for two slides to slip off from the cream, placed between them, under a load weight (70g). The time it takes for the upper glass slide to move over the lower slide to cover a distance of 10cm was noted. The spreadability was then calculated using the formula [19].

$$S = M \frac{L}{T} \quad \text{.....1}$$

M = Weight tied to the upper slide, L = Length of the glass slide, T = Time taken to separate the slides.

Skin Irritancy Test (Draize Test)

Mice skin area of up to 1cm2 was marked and shaved. Exactly 0.4g of formulation (A1-A5 and control) was applied to the specified area. It was checked for signs of irritancy such as rash, erythema or oedema at regular intervals for 24h and noted [28].

2.7 Animal Studies

Forty-nine mice were acquired at the start of the experiment from Komad Farms®, each weighing 20–35 g. For the mice paw-licking test, animals were assigned into seven groups (A1-A5, standard and control; the first five groups were composed of four mice each while the standard and control groups had two mice each. For the xylene-induced ear oedema tests, mice were divided into seven groups (A1-A5, a control, and a standard group). Each of these groups comprised three mice [29]. The animals were left to adapt to their new environment for one week before the commencement of the experiment. Proper diet (millet-whole grain and clean water), feeding and housing conditions were provided. The mice were kept in a polypropylene cage (each rat in a cage). The rats were housed under controlled temperature (24 ± 1 ° C) and relative humidity (45 ± 8%). Ethical approval was obtained for this study with approval number CMUL/ACUREC/05/23/1178 [30].

2.8 Formalin-induced nociception (Mice Paw Licking).

The topical formulations A1-A5 and control (0.1g) were applied to the right paw of mice according to their groups (A1-A5). The control formulation was applied to the right paw of the mice in the control group, while 1ml of diclofenac was administered 20 mg/kg per oral to the male mice in the standard group. After precisely one hour, 20 µL of 1% formalin was injected into the sub-plantar region of the right paw of all mice groups [31]. The mice's paw-licking activity was observed and the number of times each mouse licked its right paw in each group was recorded then the average was calculated [27]. This was done at the initial 5 min (Early Phase I—neurogenic) and between 15 and 30 min (Late Phase II—inflammatory) [32].

The percentage inhibition was determined using Equation (2).

$$\% \, Inhibition = \frac{P_c - P_t}{P_c} \times 100 \quad \text{.....2}$$

Where Pc is the average paw-licking in the control. Pt is the average paw licking in the groups A1 to A5 [27].

Xylene-Induced Ear Oedema

Topical formulations A1-A5 and control (0.1g), were administered to each animal according to their groups on the right ear. The control formulation was administered on the right ear of each animal in the control group [33]. The last group, the standard, was administered Aspirin 50mg/kg orally. After one hour, xylene was administered by topical application of 1 drop on the inner surface of the right ear of all animals in all groups. The animals were sacrificed 30 minutes later, and the left and right ears were excised [34]. The difference between the right and left ear weights was taken. The average of the difference between the right ear and left ear for each group was calculated, as well as the percentage inhibited using the formulations (3).

$$\% \text{ Inhibition} = \frac{W_c - W_t}{W_c} \quad \text{.....3}$$

Where Wc is the mean of the difference in the weight of the left and right ears for the control group, Wt is the mean difference in the weight of the ears for the formulations A1-A5 and the standard group.

2.9 Statistical Analysis

Measurements were carried out in triplicates. A one-way ANOVA test was employed to determine the statistically significant difference ($p < 0.05$). Bonferroni's multiple comparisons test (where necessary) was carried out [35].

3.0 RESULTS

3.1 Phytochemical Screening

The phyto-constituent screening of the Markhamia tomentosa extract was carried out to determine the phytoconstituents present. As shown in Table 2, the extract showed marked presence of a variety of key phytoconstituents such as flavonoids, tannins, alkaloids, saponins, cardiac glycosides, phenols, terpenoids, and anthraquinone glycosides.

3.2 Physico-chemical Evaluation

All formulations showed good homogeneity. The texture and feel were smooth for all formulations. Formulations A1-A5 had a green tint colour while the control formulation was off-white. Formulations A1-A5 had a minty *Eucalyptus* fragrance. All formulations were non-greasy. The emulsion type was evaluated using the amaranth dye test. The globules appeared red due to staining by the amaranth dye, and the continuous phase remained colourless, indicating that all formulations were water in emulsion oil. No rash, erythema, or edema was observed after the application of the formulations, implying that the formulations are safe for dermal application.

Table 2. Phyto-constituents Present in *M. tometosa* their Indicative Test, Observations and Inference.

Constituents	Test	Observation	Inference
Terpenoids	Libermann Burchard	Green color	Present
Anthraquinone glycoside	Borntegers Test	Pink color	Present
Cardiac glycoside	Keller-kilani test	Bluish green color	Present
Phenols	Phenol test	White precip-	Present
Alkaloids	Mayers test	White precip-	Present
Saponins	Foam test	Froth	Present
Tannins	Ferric Chloride	Blue black	Present
Flavonoids	Alkaline reagent	Yellow flo-	Present

3.3 pH, Microscopic evaluation, and Globule Size

The microscopic globule size is an indication of the level of stability of a formulation. Formulations with smaller globule sizes tend to be more stable. In comparison, formulations with larger globule sizes tend to coalesce and form a bi-phasic system, which is undesirable for emulsion systems. Figure 1 shows that the pH of the formulations ranged from 8.5-5.5, whilst the skin's pH is 4-6. The granular structure and intermolecular arrangement are shown in the micrograph of all formulations in Figure 2; all formulations featured small globes, which were evenly distributed.

There was absence of coalescence in the micrographs (Figure 2).

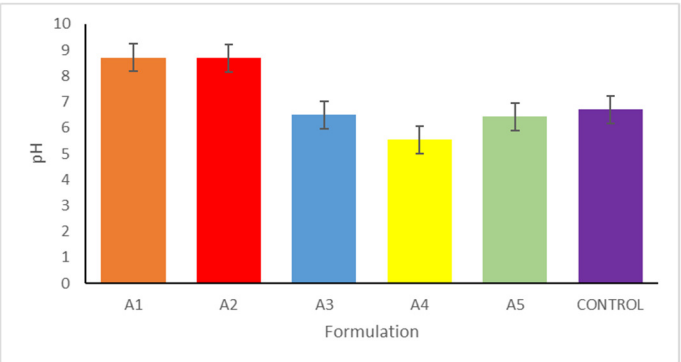

Figure 1. Potential of Hydrogen of formulations A1-A5 and the control. Results are expressed as mean ± S.D (n=3). For all data sets ($p<0.05$).

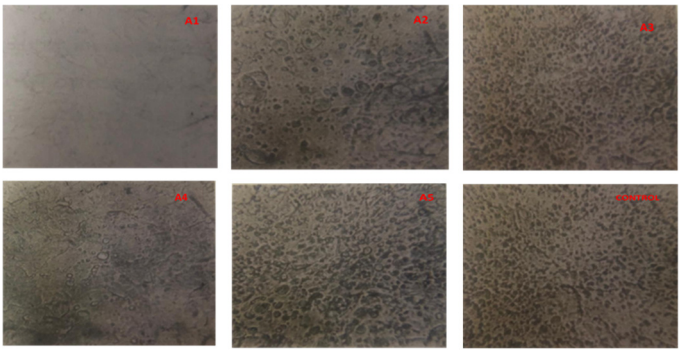

Figure 2. Photomicrographs of the Varying Formulations A1-A5 and the Control

The globule size range of each formulation is in the bar chart in Figure 3. Formulation A4 had the lowest average globule size and, hence, is the most stable of all the formulations. Formulation A2 had the largest globule size and is most likely the least stable of all the formulations.

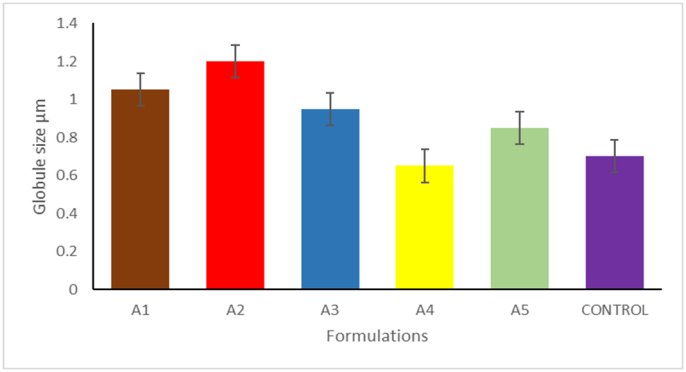

Figure 3. Average Globule Sizes of the Varying Formulations (A1-A5). Results are expressed as mean ± S.D (n=3). For all data sets ($p<0.05$).

3.4 Spreadability

Spreadability can be described as the ability of an emulsion to cover the surface of the skin easily. This depends on the molecular arrangement and viscosity of the emulsion. Emulsions with lower viscosities and fluid-like consistency have higher spreading ability, while emulsions with higher viscosity and thick consistency have lower spreadability. The formulation with the highest spreadability in Figure 4 is A4, followed closely by A1 and A5, while the formulation with the lowest spreadability was A3. The spreadability of a topical formulation is essential as it influences the degree to which the active pharmceutical ingredient in the emulsion is evenly distributed throughout the surface (site of action) of the skin.

3.5 Viscosity

The viscosity of an emulsion can be described as its degree of thickness or density; it is also the resistance level to flow. An emulsion with high viscosity will require more energy to be applied in spreading it over the site of action on the skin. All creams were pseudo-plastic and exhibited thixotropic behaviour. The fundamental molecular foundation of thixotropic behaviour depends on the alterations in intermolecular interactions under the continuous application of shear. Pseudo-plastic thixotropics show thinning behaviour when there is an increase in shear rate. In Figure 5, the formulations A1-A5 and control showed a reduction in viscosity with an increase in shear rate, indicating that the creams are easy to spread on topical application.

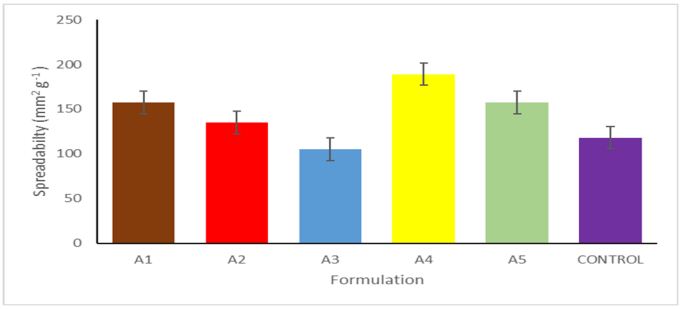

Figure 4. Spreadability of Formulations A1-A5 and the control. Results are expressed as mean ± S.D (n=3). For all data sets ($p<0.05$).

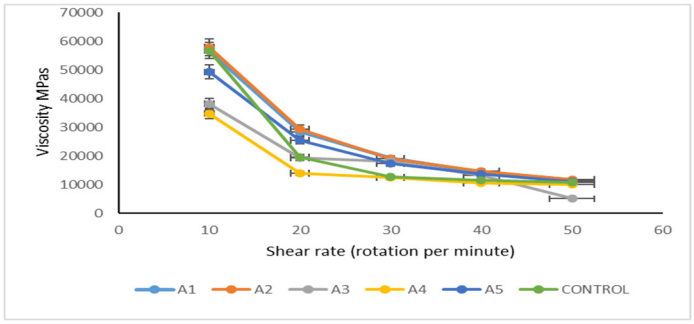

Figure 5. Viscosity Plot Showing the Effect of Shear Rate on the Viscosity of Formulation A1-5 and Control. Results are Expressed as mean ± S.D (n=3). For all Data Sets ($p<0.05$).

3.6 Anti-nociceptive and anti-inflammatory activities.

3.6.1 Inhibition of formalin-induced Nociception

Five formulations, A1-A5 and a control were tested for anti-nociceptive activities. All formulations were topically applied to the right paws of each mouse. Injection of formalin into the sub-plantar region of the right paw of the mice led to a two-phase pain response, an early neurogenic phase, and a late inflammatory phase, for both phases the level of pain was detected by paw licking. Anti-nociceptive activities were highest in formulation A1 and lowest in A5 as seen in Figure 6. The inhibition observed in A1 is comparable to the standard 20 mg/kg per oral Diclofenac sodium. No inhibition was observed

in the control formulation at the early and late phases. Formulations A3, A4, and A5 showed higher anti-nociceptive activity at the early neurogenic phase and lower activity at the late inflammatory phase.

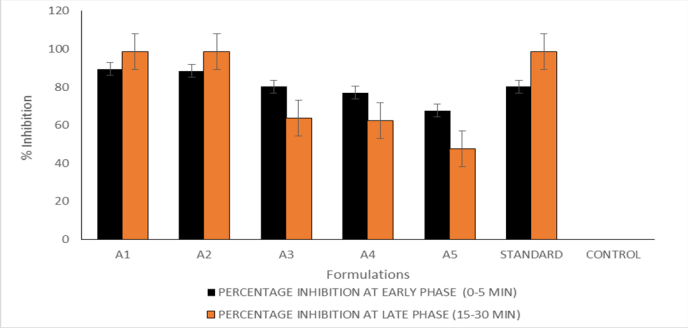

Figure 6. Percentage nociception inhibition at 5 min (Phase I—neurogenic) and between 15 and 30 min (Phase II—inflammatory) by formulations A1-5, standard and control. Results are expressed as mean ± S.D (n=3). For all data sets ($p<0.05$).

3.6.2 Inhibition of Xylene-Induced Ear Oedema

In this study, ear oedema was induced in mice by the topical application of one drop of xylene into the inner ear. Figure 7 shows that inhibition of swollenness was highest in formulations A1 and A3 (60%). The standard, which was aspirin administered at 50mg/kg orally, gave a lower inhibition of xylene-induced ear oedema (43.75%) compared to the formulations A1 and A3. The control group showed zero inhibition.

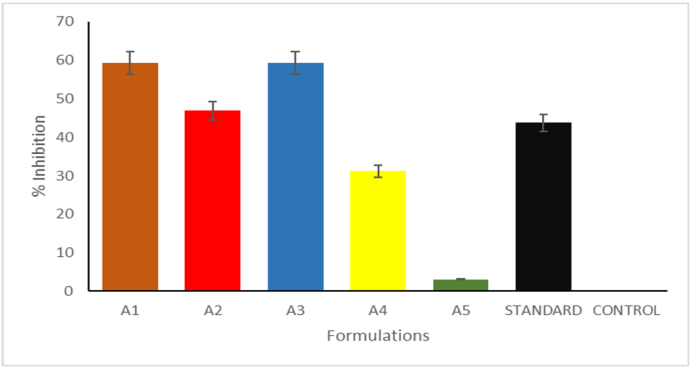

Figure 7. Percentage Inhibition of Xylene Induced Ear Edema by Formulations A1-5 Standard and Control. Results are Expressed as mean ± S.D (n=3). For all Data Sets ($p<0.05$).

4.0 DISCUSSION

The qualitative phytochemical screening of the methanol leaf extract of *M. tomentosa* reflected the presence of saponins, flavonoids, cardiac glycosides, alkaloids, steroids, and terpenoids [9]. According to scientific literature, anti-nociceptive and anti-inflammatory activities have been observed in some phyto-constituents, such as flavonoids, tannins, alkaloids, saponins, terpenoids, and steroids [10]. The anti-nociceptive and anti-inflammatory effects observed in *M. tomentosa* are likely to be due to the presence of these particular phytochemicals [36].

Eucalyptus oil, which is also a component of the formulation, has a variety of constituents such as flavonoids, alkaloids, tannin and propanoids, oxygenated sesquiterpenes, and oxygenated monoterpenes [37]. Other constituents include monoterpenes, oxygenated monoterpenes, and oxygenated sesquiterpenes such as linalool, 1, 8-eucalyptus, α- terpined and terpiene-4-ol. Studies have shown that 1, 8-eucalyptus is precisely responsible for the anti-inflammatory properties of *Eucalyptus* oil [38].

Emulsions were prepared using the fusion method to ensure adequate stability; they appeared homogenous and gave a smooth texture. These observed properties may be due to stearic acid, which acts as the emulsifying and thickening agent in the cream formulation. Stearic acid can be described as an anionic surfactant that breaks down the surface tension for oil and water phases to form the emulsion. Doing this enhances the homogeneity and smooth consistency of the cream formulations [39]. The pH of the formulations A1-A5 and control ranged from 8.5-5.5, and the skin's pH ranged from 4-6, hence the dermal safety of the topical formulations. The microscopic evaluation of the formulations showed that the water globules were uniformly dispersed in the continuous oil phase. Emulsions with smaller and more dispersed globules tend to be more stable. The formulations with the smallest globule sizes were A3 and A4; this could be because they had present in their composition the highest concentration of emulsifying agent (stearic acid).

Viscosity can be described as the resistance of an emulsion to flow or spread, while spreadability is the ability of an emulsion to spread throughout a given surface area [40, 41]. The higher the viscosity of an emulsion, the thicker the formulation; hence more energy is required to spread the formulation uniformly throughout a given surface area on the skin [42]. Carbopol and stearic acid were included as thickening agents at different concentrations in the formulations, while cetostearyl alcohol acted as a viscosity enhancer. The formulation with the least spreadability was A3, as it had the highest Carbopol concentration, while the formulation with the highest spreadability was A4, having a lower concentration of the thickener Carbopol. A4 also had the lowest viscosity, which explains why it was quickly spreadable [43]. Carbopol is a hydrophilic polymer composed of acrylic acid

monomers. It is a critical thickening agent for emulsions. The thicking effect of carbopol occurs via a gelling effect, which is activated in two phases. The first phase is the dispersion and hydration of the polymer, while the later phase involves a neutralising effect through the addition of triethanolamine.

Nociception can be described as the processing of noxious stimuli by both the central and peripheral nervous system. This triggers the activation of peripheral nociceptors as well as the nociceptive pathway. Peripheral nociceptors are nerve fibres that detect and transduce noxious stimuli as action potentials to the spinal cord and brain. Activation of the nociceptive pathway leads to an experience felt, which can be called pain [44]. Inflammation can be described as the defence or response of the human body to noxious stimuli (pain); excessive inflammatory response can lead to a major myriad of disorders such as metabolic syndrome, allergies, autoimmune diseases, cardiovascular dysfunctions, heart disease, Alzheimer's disease arthritis, depression and cancer. Non-steroidal anti-inflammatory drugs relieve inflammatory pain by inhibiting the enzyme cyclooxygenase. Cyclooxygenase is needed to transform arachidonic acid into thromboxanes, prostaglandins, and prostacyclins [45]. These molecules play a vital role in facilitating inflammation. Unfortunately, non-steroidal anti-inflammatory drugs can also cause increased blood pressure, ulcers (if taken on an empty stomach), allergies, and other skin reactions. Opioids, on the other hand produce analgesic action through presynaptic or postsynaptic pathways. Pre-synoptically, opioids act as calcium channel blockers on nociceptive afferent nerves to prevent the production of neurotransmitters. Post-synoptically, they activate potassium channels, which hyperpolarise cell membranes, causing a surge in the required action potential to develop nociceptive transmission. Opioid therapy presents the risk of drug dependence or addiction [46]. Currently, herbal products are being considered as a safer and more productive option for anti-nociceptive or anti-inflammatory action because they do not possess the pharmacological characteristics that opioids possess that cause drug dependence or addiction. To this end, *M. tomentosa* topical cream was formulated in this study with eucalyptus oil as a permeation enhancer and evaluated for its anti-inflammatory and anti-nociceptive properties. This was conducted via inhibition of xylene-induced ear oedema and formalin-induced nociception. Both procedures are behavioural techniques established to study the effects of inflammation and nociception in mice [47].

Due to complications caused by excessive pain and inflammation, inhibition of pain and inflammation is very important [48]. All formulations showed inhibition of peripheral nociception. The A1-A5 reduced the paw-licking time of the animals in a dose-dependent manner at the early phase of nociception. Inhibition of pain at the early phase was highest in formulation A1, which had the highest concentration of *M. tomentosa* leaf extract. The percentage inhibition for A1 was 89.58%, which was higher than the standard (diclofenac sodium) 80.21%, while the formulation with the lowest percentage inhibition was A5 (67.71%). Formulations with higher concentrations of *M. tomentosa* leaf extract significantly inhibited nociception. This highlights that a central analgesic activity was detected through observation of inhibition of nociception during the neurogenic phase [48]. At the late phase, 15–30 min after pain induction, formulations A1 and A2 inhibited the pain sensation almost completely, with A1 and A2 having a percentage nociceptive inhibition of 98.75%. This was the same as the percentage nociceptive inhibition of standard (diclofenac). Comparison of the control and the formulation A1 and A2 - treated groups showed a significant difference ($p < 0.05$) in the percentage nociceptive inhibition, but there was no significant difference in the nociceptive inhibition achieved with the standard drug in the (diclofenac, 20mg/kg). The high level of nociceptive inhibition seen in the late phase results from peripheral and central sensitisation mechanisms that foster termination of nociception through the accumulation of active inhibitory mechanisms that are not endogenous opioids [48,49].

Xylene triggers fluid accumulation and swelling, the expected acute inflammatory response. This leads to the production of pro-inflammatory mediators from sensory neurons that act on peripheral target cells, such as mast cells and other immune cells, producing neurogenic inflammation characterised by hyperthermia, erythema, and swelling [45]. Studies have shown that the xylene-induced ear oedema test responds well to anti-inflammatory agents by blocking phospholipase A2. The evaluation of the anti-inflammatory activity of formulations A1 and A3 showed that the formulations possessed anti-inflammatory activity as the percentage of inflammatory inhibition was higher than that of the standard [46]. Formulations A2, A4, and A5 showed lower percentage inhibition of xylene-induced ear oedema. This study also reflects that formulations A1 and A3 significantly produced the anti-inflammatory effect, most likely inhibiting the release or synthesis of inflammatory medi-

ators, prostaglandins, and polypeptide kinins, as well as phospholipase. Formulation A1 had the most potent anti-inflammatory and anti-nociceptive properties in both xylene-induced ear oedema and formalin-induced nociception experiments [50].

This current study establishes that *M. tomentosa* cream co-formulated with eucalyptus oil as a permeation enhancer exhibited anti-nociceptive and anti-inflammatory properties. Scientifically, this study has provided an evidence-based background for the exploration of the therapeutic use of the formulated emulsion for the treatment of pain in a manner completely independent of the opioid pathway.

Acknowledgements

The authors thank the Faculty of Pharmacy, University of Lagos, Nigeria, for the provision of research facilities.

Conflicts of Interest

The authors declare that there is no conflict of interests.

Authors' Contributions

IMC-D designed the animal studies, carried-out the one-way ANOVA test to detect statistically significant differences ($p < 0.05$) and bonferroni's multiple comparisons test where necessary, wrote the Methods, Results and Discussion sections of the Manuscript. **CSM** conceived and designed the protocol for preparation of emulsion/ creams, collected data generated during characterization of emulsion/cream and performed qualitative analysis of phytocostituents of *M. tomentosa.* **MOI, CPA** conceived and designed the animal studies, and co-wrote the Introduction section of the manuscript. All authors approved the final copy o f the manuscript.

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