

Assessment Of Analgesic And Anti-Inflammatory Potentials Of Topical Application Of Anti-Arthritic Cream In Wistar Rats

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Abstract

Background: There had been use of plant based herbal formulations in the management of arthritis and other related musculoskeletal system without scientific investigation.

Aim: The study assessed analgesic and anti-inflammatory potentials of topical application(TA) of anti-arthritic cream in Wistar rats.

Material and Method: Thirty six (36) animals (18 mice and 18 rats) were used for the studies. The animals were divided into two study Groups (A and B) (n= 6) per group for the evaluation of analgesic and inflammation studies. The experimental protocol for analgesic studies is viz: Control Group AI received TA of 0.2 mL saline solution on the shaved hindlimb, Group AII received TA of anti-arthritic cream, Group AIII received TA of cream base and Group AIV received TA of Diclomol cream. Analgesic activity was evaluated in mice by inducing 0.6% acetic acid after 1 hour application of the test sample and number of writhing were determined. While experimental protocol for inflammation is viz: Control Group BI received TA 0.2 mL saline solution, Group BII received TA anti-arthritic cream, Group BIII received TA cream base and Group BIV received TA Diclomol cream. Anti-inflammation activity was evaluated in rats by carrageenan induced paw licking edema in Wistar rat.

Result: Result showed that anti -arthritic cream and Diclomol showed identical 100% inhibitions in the number of abdominal writhing in male mice and higher percentage of inhibition than Diclomol in anti-inflammatory activity.

Conclusion: The study revealed that topical application of anti-arthritic cream possesses analgesic and anti-inflammatory activities.

Keywords: Anti-arthritic cream, analgesic, anti- inflammatory

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I. Introduction

Arthritis was a term introduced by Sir Alfred Garrod (1859) to describe a chronic inflammatory disease of peripheral joints which he distinguished from other related arthritic pain such as acute rheumatoid, and gout. Arthritis is also seen as a group of disorder affecting the joints which comprises more than 50 clinical conditions arising from degenerative, infiltration of inflammatory cells (monocytes), synovial hyperplasia, bone erosion, narrowing of the joint space, infective metabolic or autoimmune causes and these include osteoarthritis, rheumatoid arthritis (RA), spondylitis and many others forms of arthritis.

The common arthritis symptoms include swelling, pain, stiffness, decreased range of motion and the decrease in mobility makes it difficult for a person to remain physicallyn8 inactive, thereby contributing to an increased risk of obesity and vulnerability to cardiac disorder. According to World Health Organization about 350 million people suffers from arthritis annually. Unlike before when arthritis is rare among the African black people, now people of all ages, sexes, and races could have arthritis and it is leading to cause of human joints. In Nigeria, arthritis is now a significant medical condition and the Nigerian Orthopaedics Association disclosed that 15 million are suffering from arthritis annually which is the leading cause of disability with both physical and emotional impacts. The common risk factors are family history, predisposition, age, previous injuries to the joint and obesity. The prevalence of arthritis is higher among women (28.3%) than men (18.2%). Nearly 294,000

children under the age of 18 are affected by juvenile arthritis [1], it also affects more than half of adults with diabetes and heart disease [2]. The prevalence of RA is generally lower in developing countries, with few or no cases found in some African [3]. According to [4] reports on study in urban living in Soweto showed a prevalence of RA among urban blacks equivalent to that in white Europeans, while rural black groups have showed much lower prevalences [3, 5], while osteoarthritis affecting an estimated 21 million adults in the United States (NIAMS). Arthritis has a profound economic, personal and social impact in the United States of America. In 2003, arthritis conditions cost the U.S. economy \$128 billion (MMWR 2007). In the United Kingdom, the estimated annual cost of arthritis to the NHS is between £240 M and £600 million per annum and in 2013, United States Bone and joint Initiative stated that the total national arthritis attributable medical care cost is \$305.5 billion or 1% of USA 2013 Gross Domestic Product.

The treatment of Arthritis generally involves a multimodal approach. Pharmacological treatment/management is used to control inflammation together with physiotherapy and surgery which includes the administration of analgesics and non-steroidal anti-inflammatory drugs, but their use does not provide adequate pain relief in most patients. Treatment modalities for arthritis are mostly symptomatic although recently it has been suggested that use of disease-modifying anti rheumatic drugs has led to important gains in the overall ability to treat patients, resulting in a better health status for patients with arthritis. The limitation in the use of conventional analgesic and anti-inflammatory drugs for the treatments and management of arthritis include exorbitant costs and adverse effects, expensive investigations, joint repairs, joint replacement, surgeries and inability to permanently resolve the cases hence the need for alternative approach/therapy.

Plants have been used thousands of years in Africa, China and other Asian countries for the treatment of arthritis and related musculoskeletal disorders. Based on several research works, more than 100 plants have been discovered to have pain-relieving, anti-inflammatory and anti-oxidant properties. According to Chinese herbal theory, interactions among the different herbs in a formula exert a synergistic effect and neutralize potential toxicity and side effects of the individual constituents [6]. Plants are usually accessible, affordable, culturally, traditionally and sociably acceptable and easy to prepare, hence a lot of people prefer taking them to the more highly priced orthodox medicine and more so, botanical medicine are important part of the culture and tradition of African people. Therefore this study investigated analgesic and anti - inflammatory effects of topical application of anti - arthritic cream in Wistar Rats.

II. Materials And Methods

Plant materials

Oils extract from the *Mentha piperita*, *Zingiber officinale*, *Capsicum frutescens*, *Glycyrrhiza glabra* and cream base were purchased from Herbarium essential oil organic shop at Ojota, Lagos Nigeria and used for the formulation of the anti-arthritic cream

Ethical Approval

Ethical approval was obtained from College of Medicine Health Research Ethics Committee with approve Number CMUL/HREC/10/19/629

Phytochemical Screening of the Oils extract from the plant materials

Oils extract from the *Zingiber officinale*, *Capsicum frutescens*, *Glycyrrhiza glabra* were analyzed using gas chromatograph and mass spectrometer (GCMS, QP 5050A Shimadzu, Japan), equipped with GC-17A, HP 5MS (5% phenyl methylsilane). A capillary column (30 m x 10.25 mm, i.d., 0.25 µm film thickness) was used with a HP5972 mass selective detector and an electron ionization system with ionization energy of 70 eV. Helium then was used as the carrier gas at a flow rate of 1 mL/min keeping constant inlet pressure at 200 p.s.i. Injector and MS transfer line temperatures were set at 220 °C and 290 °C respectively. The gas chromatography column temperature was gradually increased from 40 °C to 240 °C at a rate of 3 °C/min with a hold time of 10 min followed by final increment up to 250 °C at a rate of 10 °C/min. 1 µL of diluted samples (1/100 in acetone) were injected manually and split less.

The constituents of Oils extract from the *Zingiber officinale*, *Capsicum frutescens*, *Glycyrrhiza glabra* were identified by using standards of the main components comparing their relative retention times and matching the mass spectra fragmentation pattern with those of NBS75K library data stored in the GCMS database [7].

Formulation of anti-arthritic cream

<i>Mentha piperita</i> oil	=	12.8 %
<i>Zingiber officinale</i> oil	=	30.0 %
<i>Capsicum frutescens</i> oil	=	40.0 %
<i>Glycyrrhiza glabra</i> oil	=	17.2. %

All in cream base to give final formulation

Animals

Forty eight (48) male animals (i.e Twenty four (24) Wistar rats weighing from 120 g-130 g and male mice weighing from 25 g $\pm$ 28 g) bred and housed in the animal facility center of the Nigeria Natural Medicine Development Agency (NNMDA) were used for the evaluation of analgesic and anti-inflammatory studies. The animals were allowed to acclimatize for one week before the commencement of the study and kept in a photo period controlled environment (12 hours light-dark cycle) all the mice were kept in cages with solid floors covered with wood shavings and were allowed access to food and water ad libitum. The animals were identified by Dr. Amaeze N.H in Department of Zoology, Faculty of Science, University of Lagos with a reference number 002/2021.

Experimental Protocol

Acetic acid- induced writhing in mice(Analgesic Activity)

Male mice hind limb fur in the study groups were shaved with a new and sterilized razor blade under chloroform as anaesthetic agent to expose their skin for daily topical application of anti-arthritis cream. The mice were divided into four study groups (n=6 per group) in this order: Control Group AI: received 0.2 mL topical application of saline water on the shaved hind limb portion with soft brushes, Group AII: received 0.5 g topical application of cream base on the shaved hind limb portion with soft brushes, Group AIII: received 0.5 g topical application of anti- arthritis cream on the shaved hind limb portion and Group AIV received 0.5 g of Diclomol (positive control). One hour after topical application of the test samples, 0.6 % acetic acid was injected and the cramp performed by each mouse were immediately counted for 10mins. The effect of the test materials on the pain induced by 0.6 % acetic acid was evaluated according to the method of [8]. It consists in causing pain in the mouse by intra peritoneal injection of 0.6 % acetic. Infact, acetic acid causes five minutes after injection, abdominal pain which manifests itself by torsions of the dorso-adominal muscles or cramps.

The analgesic activity was expressed as percent inhibition.

$$\% \text{ Inhibition} = \frac{\text{Co} - \text{Ct}}{\text{Co}} \times 100$$

Where, Co = control, Ct = tests

Carrageenan-induced paw oedema(Anti-inflammatory activity)

Male Wistar rats hind limb fur in the study groups were shaved with a new and sterilized razor blade under chloroform as anaesthetic agent to expose their skin for daily topical application of anti-arthritis cream. The male Wistar rats were divided into four study groups (n=6 per group) in this order: Control Group BI: received 0.2 mL topical application of saline solution on the shaved hind limb portion with soft brushes, Group BII: received 0.5 g topical application of cream base on the shaved hind limb portion with soft brushes, Group BIII: received 0.5 g topical application of anti- arthritis cream on the shaved hind limb portion and Group BIV: received 0.5 g of Diclomol (positive control). One hour after topical application of the test samples Pedal inflammation in male Wistar rats was produced by injection of 0.1ml of 1% Carrageenan into the right hind foot of the rat under the supplantar aponeurosis as described by [9].The inflammation was quantified by measuring the volume (oedema) displaced by the paw. Measurement was done by wrapping a piece of cotton thread round the paw size and the circumference was measured using a metre rule.

The inhibitory activity was calculated according to the formula:

$$\% \text{inhibition} = \frac{[(\text{Ct} - \text{Co}) \text{ control} - (\text{Ct} - \text{Co}) \text{ test}]}{(\text{Ct} - \text{Co}) \text{ control}} \times 100$$

Where:

Ct- = mean paw size in the treated group

Co = mean paw size in the control group

Statistical Analysis

Data are represented as the Standard Error Mean (SEM) of the different experiments under same conditions. Comparisons were done with the use of one ways Analysis of Variance (ANOVA) within the groups. The results with the $P < 0.05$ were considered significant.

III. Results

Effect of topical application of anti-arthritic cream, cream base and diclomol on analgesic activities in Wistar rats

Figure 1 show that abdominal writhing occurred in Group III (cream base) upon topical application of cream base on the shaved portion hindlimb in mice compared to the Control Group AI with 8.6% inhibition (Figure 2). While in Group AII (anti-arthritic) and Group IV Diclomol) there were reductions in the abdominal writhings in Mice with the 100% maximum inhibitions upon topical application of anti-arthritic cream and diclomol on the shaved portion of the hindlimb in Mice compared to the Control Group AI ($p < 0.05$ in each case). Meanwhile, the number of writhing exhibited in Group AIII (cream base) was similar to that exhibited in Control Group AI. But there was no significant difference when compared with each other ($p < 0.05$). Percentage writhing inhibitions showed that both Group AII (anti-arthritis) and Group AIV (Diclomol) exhibited 100 % inhibition of pain and Group AIII (cream base) exhibited 8.6 % inhibition of pain (Figure 2) compared to the Control Group A1 ($p < 0.05$ in each case). Moreso, both Group II and Group IV exhibited high inhibitions of writhing but Group III exhibited low inhibition compared each other

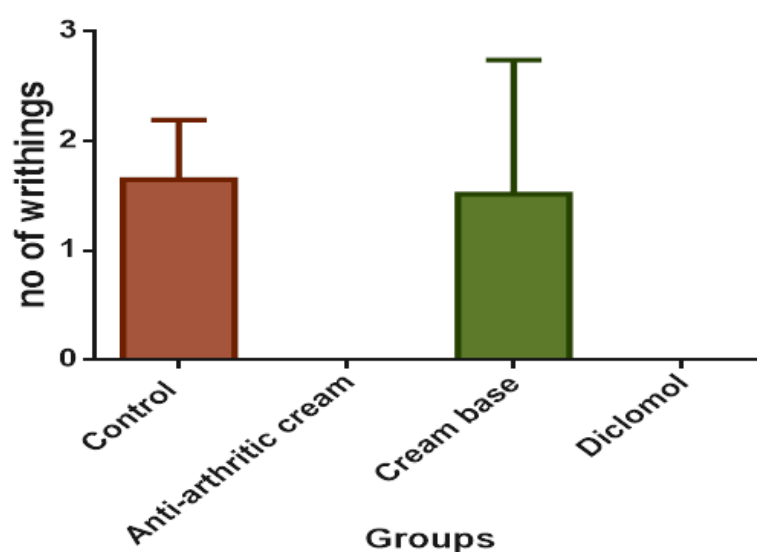


Figure 1: show effect of topical application of anti-arthritic cream, cream base and diclomol on the number of writhing produced by the interperitoneal injection of 0.6% acetic acid in albino mouse. The results are expressed as a mean standard error $n = 6$ rats per group. * $p < 0.05$ compared to the Control Group 1

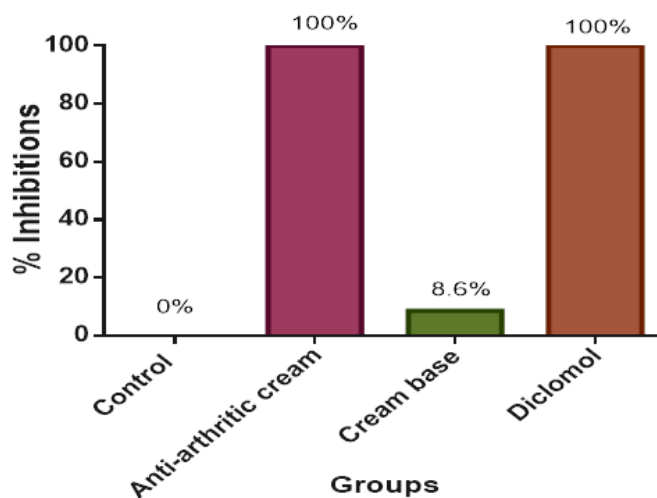


Figure 2: show effects of percentage inhibition of topical application of anti-arthritic cream, cream base and diclomol on writhing's produced by intraperitoneal induction of 0.6% acetic acid in albino mouse. The results are expressed as a mean standard error $n = 6$ rats per group. * $p < 0.05$ compared to the Control Group.1

Effect of topical application of anti-arthritis cream, cream base and diclomol on anti-inflammatory activities in Wistar rats

Figure 3: show that in Groups BII, BIII and BIV there was a change in the mean edema formation upon topical application of test substances(i.e anti-arthritis cream, cream base and diclomol on the shaved hindlimb portion in Wistar rats) compared to the Control Group B1. There was increase in the edema formation in Group BIII, while in Groups (BII and BIV), there was slight decrease in the edema formation compared to Control Group B1. The decrease was not significantly difference compared to each other. The percentage inhibition of edema formation in Group BII and Group BIV (figure 4) were reduced (-50 % and -10.5 %), while in Group III there was edema formation inhibition up to 8.8 % compared to Control Group B1

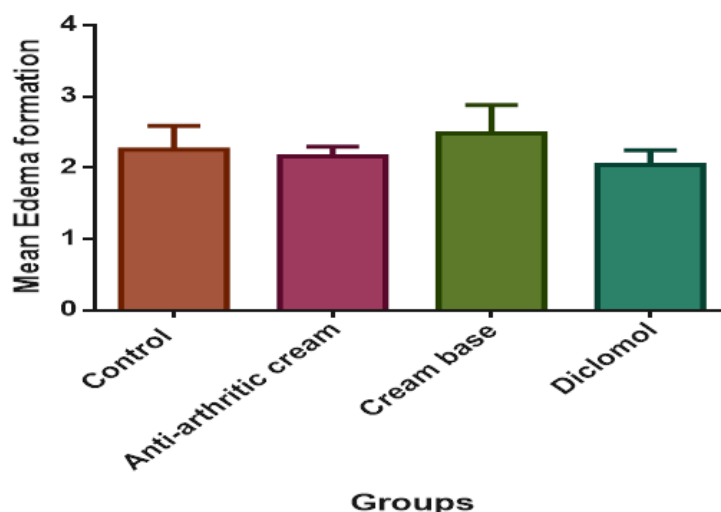


Figure 3: effect of topical application of anti-arthritis cream, cream base and diclomol on the mean induced edema formation in Wistar rat. The results are expressed as a mean standard error n= 6 rats per group. *p < 0.05 compared to the Control Group

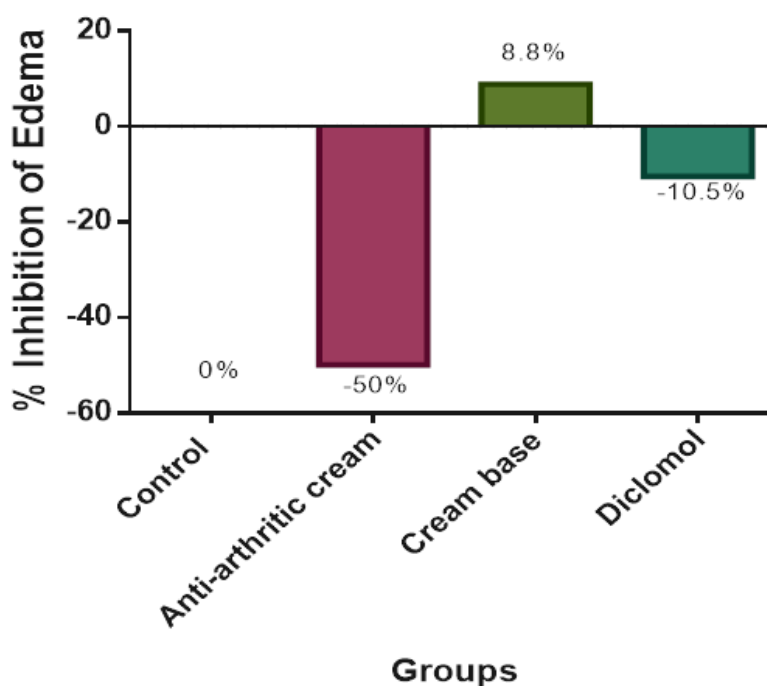


Figure 4: effect of percentage inhibitions of topical application of anti-arthritis cream, cream base and diclomol on the mean induced oedema formation, in rats. The results are expressed as a mean standard error n= 6 rats per group. *p < 0.05 compared to the Control Group 1.

IV. Discussion

Analgesic and inflammation of topical application of Anti-arthritis cream formulated from *Mentha piperita* oil, *Zingiber officinale* oil, *Capsicum frutescens* oil and *Glycyrrhiza glabra* oil was investigated in animal model. The acetic acid induced writhing is a visceral pain model are widely used for the evaluation of peripheral nociceptive activity. The intraperitoneal administration of the agent that irritates the serous membranes cause a stereotypical behaviour in mice which is characterized by abdominal contraction, movement of the body as a whole, twisting of the dorsal abdominal muscles and a reduction in the motor activity and coordination [10]. Findings from the present study showed that topical application of anti-arthritis cream possesses analgesic property with 100% pain inhibition and this might be due to the presence of capsaicin and gingerol and other lesser active compounds in the formulation. It has been reported that long term exposure to capsaicin results in the loss of pain sensation in human.

These analgesic activities of anti arthritis cream is similar to the analgesic activities of Diclomol with 100% pain inhibitions. This indicates a marked evidence of analgesic property of anti-arthritis cream. Acetic acid causes an increase in the peritoneal fluid level of prostaglandins lipooxygenase production which partially involves peritoneal receptors and inflammatory pains by inducing capillary permeability[11]. Acute anti-inflammatory activity of topical application of anti-arthritis cream was performed using carrageenan induced oedema formation test. Carrageenan induced oedema formation is an excellent representatives model of acute inflammation [11] indeed carrageenan is a polysaccharide which injects into the animal and induces local inflammation characterized by increased vascular permeability, edema, and extravasation of neutrophils (Gamache *et al.*, 1986), the biphasic action of carrageenan involves the release of amino vaso-active mediator in the first phase between 30 min to 1 hour while second phase mediated by prostaglandin release after one hour administration of carrageenan. The kinins intervene between the two phase [12]. The results from topical application of anti-arthritis cream and diclomol produced significant increase in the oedema formation in the rats paw (inflammation). Oedema formation in anti-arthritis cream and diclomol were identical. This acute oedema formation could be explained as the release of inflammatory agents such as histamine and serotonin. More so, the anti-inflammatory effects of anti-arthritis cream in comparison with the diclomol could be better explained in terms of their percentage inhibition of the oedema formation. Anti- arthritis cream inhibits the oedema formation than the diclomol as noticed. Therefore, anti-arthritis and diclomol have strong affinity to reduce the effect of inflammation agents.

V. Conclusion

The study revealed that topical application of anti-arthritis cream possesses analgesic and anti-inflammatory activities.

Conflict of interest = No conflict of interest

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