

RESEARCH ARTICLE

GC-MS ANALYSIS AND EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF THE OIL FRACTION OF METHANOL EXTRACT OF *OECOPHYLLA LONGINODA* (WEAVER ANT) IN WISTAR RATS

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ABSTRACT

The present study was aimed at investigating oil profile and the anti-inflammatory activity of methanol extract of the African weaver ant, *Oecophylla longinoda* (OL) using three models in wistar rats. For the dextran, carrageenan and formaldehyde test models: 0.1 mL of 1.5% w/v dextran; 0.1 mL of carrageenan (1% w/v) and 0.2 mL of formaldehyde (1% w/v) were each injected into the sub plantar tissue of the hind paw of the rats respectively to induce inflammation while OL was administered orally at a dose of 200 and 400 mg/kg. Indomethacin (10 mg/kg) and Tween 80 (10 ml/kg) served as positive and negative standards respectively. The major components detected from the oil fraction were carvone (Retention time (Rt): area percent: 13.547: 21.12%) a terpenoid and oleic acid (32.273:7.16%), an unsaturated fatty acid. The extract showed a notable reduction in inflammation in each of the models, ($p < 0.01$) by decreasing the mean paw size of the rats after treatment in comparison to the negative control. The 200 and 400 mg extract in the three models showed significant inhibition of inflammation. These data suggest that OL had a positive anti-inflammatory effect on both acute and sub-chronic inflammation.

KEYWORDS

Oecophylla longinoda, pain, dextran, anti-inflammatory, gas chromatography mass spectrometry.

1. INTRODUCTION

In recent years, there has been a growing interest in the potential medicinal properties of insects. Among these insects, the *Oecophylla longinoda* ant has gained attention due to its unique physiological and chemical composition. In traditional medicine, the crushed body of *O. longinoda* ants has been used topically to relieve inflammation associated with conditions like rheumatism, arthritis, and muscle sprains (Iyekowa et al., 2022; Adeleke et al., 2018). Research studies have shown that the *O. longinoda* possesses anti-inflammatory activity and is reported to also exhibit antinociceptive properties, which reduce the sensitivity to painful stimuli. By inhibiting the production of prostaglandins and other inflammatory -related mediators, it effectively reduces inflammation and provides relief (Momo et al., 2023). Studies have revealed that *O. longinoda* ants possess potent anti-inflammatory activity. The formic acid present in these ants has demonstrated the ability to suppress the production of pro-inflammatory cytokines and chemokines, thereby reducing the inflammatory response. This property makes *O. longinoda* extract potential candidate for the development of novel anti-inflammatory drugs.

An essential process that helps organisms respond to potentially harmful stimuli/ response is inflammation, it is a host defense process that is triggered (Ferrero-Miliani et al., 2007). It is a helpful process because it offers a physiology that is conducive to excluding unwanted stimuli or invading microorganisms. Yet, regulation of the process is necessary because uncontrolled inflammation is responsible for the pathophysiology of diseases like cancer (Jiang et al., 2023). Inflammation

is a frequent occurrence in many acute and chronic debilitating diseases and is a key contributor to morbidity in the age of contemporary lifestyle. Inflammation, along with respiratory, autoimmune, and cardiovascular illnesses, leads to rheumatoid arthritis, diabetes, cancer, Alzheimer's disease, and atherosclerosis if left unchecked. A complex network of many mediators, a wide range of cells, and the execution of numerous pathways all play a part in inflammation (Jiang et al., 2023). The fact that the immune system and inflammatory processes are implicated in a wide range of mental and physical health issues that account for the majority of morbidity and death in the modern world is among the most important medical breakthroughs in the previous 20 years (Bennett et al., 2018). Indeed, chronic inflammatory diseases are now known to be the leading cause of death worldwide, accounting for more than 50% of all fatalities. Some of these conditions include autoimmune and neurodegenerative diseases, ischemic heart disease, stroke, cancer, diabetes mellitus, chronic kidney disease, and non-alcoholic fatty liver. There is growing evidence that an early development may be associated with an increased risk of chronic inflammation, that it's impacts can also alter adult health and mortality risk across the lifespan (Lee et al., 2024)

Activated immune system, including that of immune cells and biomolecules, is linked to inflammatory conditions (Renz et al., 2017). A tissue becomes inflamed as a protective measure against invasion by bacteria, parasites, and viruses. Acute inflammation is the initial rapid response to infection, tissue necrosis, foreign bodies. Inflammation is characterized by redness, heat, swelling, and loss of function which are as a result of increased vascular permeability, increased blood flow, and

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nerve fiber sensitization. Chronic inflammation is a prolonged response to offending agents (Renz et al., 2017). Inflammation is a beneficial host response to foreign invaders and necrotic tissue, but it may also cause tissue damage, when the mediators released begin to attack the body, that is why the need for new drugs which can reduce inflammation to the barest minimum cannot be overemphasized.

The inflammatory process begins with an acute phase which is a rapid response to the offending agents that lasts for a short duration. Acute inflammation functions as a homeostatic mechanism that aids the host's healing process (Gemole et al., 2018). An established method for assessing the effectiveness of acute anti-inflammatory drugs is carrageenan-induced paw edema (Mansuori et al., 2015). Rats that are injected with it experience two distinct periods of inflammation: the early phase and the late phase. The first phase, which lasts for about an hour, is marked by the release of pre-generated inflammatory mediators such as histamine, serotonin, and bradykinins. The early phase mediators begin to trigger events that result in neutrophil infiltration and further prostaglandin production by cyclooxygenases (COX) throughout the first hour, this is quickly followed by the late phase (Gilligan et al., 1994). Other mediators that play a role on proinflammatory cytokines like interleukin-1b (IL-1b) and tumor necrosis factor- α (TNF- α), as well as neutrophil-derived free radicals and nitric oxide (NO), are present in the late stage of carrageenan-induced inflammation. The persistence of late phase mediators causes inflammation to expand into the chronic phase, which leads to inflammation-related diseases. (Gilligan et al., 1994). Another model for studying inflammatory process is the dextran-induced paw edema model. This is characterized by increased vascular permeability which allows for increased blood flow, migration of cells and molecules of the immune system into the surrounding tissues. This leads to the release of chemical mediators such as kinins, histamine and serotonin, leading to osmotic

edema with low levels of protein and neutrophils (Abdulkhaleq et al., 2018). The formaldehyde induced edema model results from a neurogenic inflammation mediated by neuropeptides. It is typically simpler to distinguish between the response from grooming when formalin is injected into the dorsum or plantar tissue of a hind paw rather than a forepaw. In any case, the injection results in licking, flinching, shaking, and favoring of the affected paw, which normally happens in two phases, the first of which can continue up to 10 minutes after injection and the second of which can last for between 20 and 60 minutes.

The synthetic anti-inflammatory medications already on the market may be in the majority, but their potential for toxicity cannot be completely disregarded. Nonsteroidal anti-inflammatory medications (NSAIDs) and corticosteroids have both been created, however investigations on their safety profiles have indicated that none of them are categorically safe. Herbal remedies have returned to meet our essential healthcare needs as a result of the unfavorable side effects of synthetic and chemical medications, which include gastrointestinal discomfort and the recurrence of symptoms after withdrawal process (Nathaniel and Israni-Winger, 2020). Therefore, this study was aimed at evaluating the anti-inflammatory effect of OL using dextran induced hind paw edema, carrageenan induced hind paw edema and formaldehyde induced edema models.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

The ants were collected from an almond tree (*Terminalia catappa*) in Ikpoba town in Edo State, Nigeria. Identification was done by a zoologist in the Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria,



Plate 1: The African Weaver Ant; *Oecophylla longinoda* (Tailor ant)

2.2 Solvent Extraction

Eighteen ant nests were gathered and immersed in distilled water to immobilize the ants and separation from the leaves by sorting. The ants were then crushed fresh in a mortar. Over an 8-hour period, 73.8 g of crushed ants were totally extracted in a Soxhlet extractor with 750 mL of methanol and the crude extract was concentrated in a rotary evaporator (model RE, 200 USA) at 50°C.

2.3 Fractionation of the crude extract

The crude extract was made polar by mixing methanol and distilled water in a 9:1 mL ratio respectively. This was fractionated further by using a solvent system of methanol: hexane in ratio 4:6 to achieve an oily fraction upon concentration with rotary evaporator.

2.4 Experimental Animals

Mature wistar rats weighing 25-35 g of either sex were obtained from the Department of Pharmacology and Toxicology, Faculty of Pharmacy animal house, University of Benin. The animals were housed in temperature-controlled rooms (25°C), with a 12:12 hour light/dark cycle and free access to food and drink. The animals were kept in plastic cages with bedding made of sawdust. The animals were given two weeks to adapt before being used. The procedures presented were reviewed and approved by the Ethical Committee on the use of Experimental animals, Faculty of Pharmacy, University of Benin.

2.5 Animal study

The rats were divided into four groups of five each for the three different

experimental models. The first group was given tween 80 (10 mL/kg) as a negative control. As a positive control group, the second group received Indomethacin (10 mg/kg). The oil fraction of OL was given to the third group at a dosage of 200 mg/kg while the fourth group got a dose of 40 mg/kg. The treatments were administered orally via an orogastric tube.

2.6 Ethical considerations:

Permission to conduct the current study was obtained from the Faculty of Pharmacy, Animal Use and Ethics Committee of the University of Benin with a permit reference number EC/FP/022/23.

2.7 Preliminary Bioactive Chemical Screening

To assess the presence or absence of bioactive components from the methanol extract of OL, preliminary bioactive chemical screening for secondary metabolites were performed by standard methods. The presence of saponins, alkaloids, phenolics, flavonoids, glycosides, terpenoids, and eugenols were determined by qualitative color changes in test reagents. This information will give an indication of the possible chemical constituents class responsible for the anti-inflammatory effects.

2.8 Estimation of chemical constituents by GC-MS

The oil fraction constituents were determined using a GC (Agilent 7890B) equipped with an HP-5 ms ultra-inert column and linked to a mass spectrometer (Agilent 5977A). The substance was dissolved in additional methanol before analysis.

2.9 Carrageenan-induced paw edema

The effect of OL on acute inflammation was evaluated in wistar rats using

modified carrageenan-induced hind paw edema model (Abdelrazzag et al., 2015). Male healthy rats weighing (150-280 g) were divided into four groups (n=5). Group (I) received Tween 80 orally (10 mL/kg) this dosage served as the negative control. For group (II) animals, they received indomethacin (10 mg/kg) orally and served as the positive control, while Groups (III) and (IV) received 200 and 400 mg/kg of OL respectively orally. Sixty minutes after the oral administration of drugs/extracts, induced inflammation by injecting 0.1 ml of carrageenan suspension (1% w/v) each rat's right hind paw's subplantar tissue received this diluted in normal saline. Each rat had its paw size measured separately by means of a vernier caliper prior to treatment, after carrageenan at hourly basis for 4 hours (Mansouri et al., 2015). The mean of the paw size observed in the groups treated with extract were contrasted with those in the control. The mean inflammatory value observed for the negative control group (Tween 80) was considered as 100%. This same process was repeated for **dextran induced hind paw model** except for the specific dosage of 0.1 ml of 1.5 % w/v dextran in normal saline while other dosage of test samples and controls were constant.

2.10 Formaldehyde induced hind paw edema

The effect of OL on sub-acute inflammation was investigated by using the formaldehyde-induced hind paw edema. Male rats weighing (150-280g) that were healthy were divided into four groups (n=5). Group (I) received Tween 80 orally (10 mL/kg) which served as a control. Group 2 received indomethacin (10mg/kg) orally and served as the positive control, while Groups (III) and (IV) received 200 and 400 mg/kg OL respectively orally. Doses of OL, indomethacin as well as Tween 80 were administered once a day for a period of 2 days. An hour after the last dose was administered; 0.2 ml of formaldehyde (1% w/v) was injected into the rat right hind paw. Prior to formaldehyde injection, the paw volumes for each rat were

measured via a vernier caliper separately. Paw volumes after formaldehyde administration were measured at 3, 6 and 24 hours on the first day and thereafter measured daily till the 14th day. The anti-inflammatory activity of the extract was determined by comparing the mean paw size with that of the positive control (indomethacin) and negative control (Tween 80). The mean value obtained by the negative control was considered as 100% (Mansouri et al., 2015).

2.11 Evaluation of acute toxicity

The acute toxicity determination was conducted by using modified method approach to practical acute toxicity testing (Ogbeide et al., 2022).

2.12 Statistical Analysis

Data were presented as mean \pm standard error mean (SEM). The results were analyzed using Statistical Package for the Social Sciences (SPSS) version 19. Statistical significance was determined by Student's *t*-test and *P* value less than 0.05 was considered as significant. Differences among the groups calculated by one-way analysis of variance (one way-ANOVA) followed by Dunnett's post hoc test. A *p* value of less than 0.05 was considered significant.

3. RESULTS AND DISCUSSION

3.1 Bioactive chemical constituents

Glycosides, saponins, terpenoids and phenolics were among the chemical constituents detected.

The bioactive chemical constituents present in the oil fraction of the ants are shown in Table 1.

Table 1: Bioactive chemical constituents in oil fraction of *Oecophylla longinoda* (Tailor ant)

S/N	Bioactive chemical constituents	Oil fraction (Tailor ant)
1	Glycoside	+
2	Saponin	+
3	Phenolics	+
4	Tannins	-
5	Eugenol	+
6	Steroid	+
7	Terpenoids	+
8	Alkaloids	-
9	Flavonoids	+

Key: - = absent , + = present

In this research, the presence of flavonoids, steroids, phenolics, terpenoids and alkaloids corroborates the findings of researchers with methanol extract of whole part of weaver ants (Omonmehlen and Iyekowa, 2023). In a related study, the presence of essential oils and terpenoids have been reported as bioactive chemical constituents in *O. longinoda* (Kempraj et al., 2020). These general class of steroids and terpenoids have been classified as major chemical constituents playing huge role as trail,

sexual, alarm and defense pheromones in insects species (Igwe and Eze, 2015).

3.2 GC-MS Analysis of oil fraction of *O. longinoda*

The GC-MS chromatogram (Figure 1) showed 53 peaks which indicate from the chemical abstract service fifty-three chemical compounds. The compounds identified in the oil fraction are thus presented in Table 2.

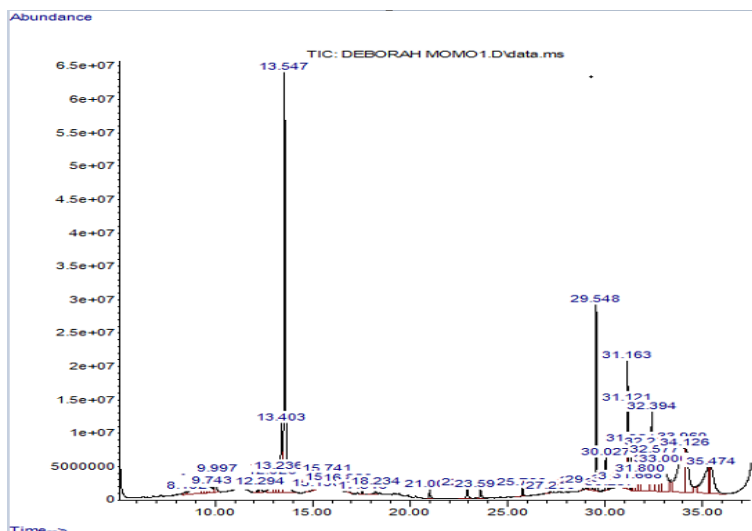


Figure 2: GC-MS chromatogram of oil fraction of OL

Table 2: GC-MS Analysis of Ethyl acetate fraction of *O. Longinoda*

S/N	Retention Time (Rt)	Name of the Compound	Molecular Weight (g/mol)	Peak area (%)
1	8.492	9-Tetradecenal, (Z) Pheromone	210.3	0.24
2	9.165	Cis-9-Tetradecenoic acid, isobutyl ester	282.5	2.35
3	9.329	Arginine (Vasodilator)	174.2	0.32
4	9.497	Heptadecanolide	268.4	0.39
5	9.563	2-Methyl-Z, Z-3, 13-octadecadienol	280.5	0.14
6	9.695	Ethylborane, B, B-di(ethylamino)-	128.0	0.45
7	9.743	2-Heptadecenal	252.4	0.63
8	9.997	Octanoic acid, methyl ester	172.2	0.99
9	12.162	Methyl 10, 11 - octadecadienoate	294.5	0.10
10	12.294	Cyclohexanol, 2-methyl-5-(1-methylethenyl) -	154.2	0.36
11	12.926	Octanoic acid, methyl ester	172.2	1.27
12	13.021	N-Acetyl-d-serine	147.1	1.66
13	13.236	Isopropene benzene	117	1.60
14	13.403	D - Carvone	150.2	4.31
15	13.547	(-)-Carvone	150.2	21.12
16	14.968	Arginine	174.2	0.02
17	15.080	3-Butenamide	85.1	0.02
18	15.190	6-Octadecenoic acid, methyl ester, (Z) -	296.4	0.02
19	15.741	Decenoic acid, methyl ester	184.2	0.05
20	15.854	(-)-8-p-Menthen-2-yl, acetate, trans	196.2	0.18
21	16.829	2 - Cyclohexen-1-ol, 2-methyl- 5-(1-methylethenyl)-, acetate, (1R-cis)-	194.2	0.34
22	17.546	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]	204.3	0.12
23	18.234	Aromandendrene	204.3	0.27
24	21.004	Dodecanoic acid, methyl ester	214.3	0.32
25	22.919	Carotol	222.3	0.53
26	23.598	Apiol	222.2	0.42
27	25.766	Methyl tetradecanoate	242.3	0.28
28	27.208	Cyclododecane, ethyl-	196.3	0.10
29	28.854	N-Acetylmannosamine	221.2	0.05
30	29.007	Cetene	224.4	0.13
31	29.265	Oxacyclotetradecane-2,11-dione,13-methyl-	240.3	0.35
32	29.548	Hexadecanoic acid, methyl ester	270.4	4.65
33	30.027	Dibutyl phthalate	278.3	0.87
34	30.237	Z-11-Tetradecenoic acid	254.4	0.02
35	30.563	Oleic Acid	282.4	0.02
36	30.939	13-Tetradecen-1-ol acetate	268.4	0.39
37	31.121	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	294.4	1.53
38	31.163	9-Octadecenoic acid (Z)-, methyl ester	296.4	3.00
39	31.351	Methyl stearate	298.5	0.95
40	31.492	cis-Vaccenic acid	282.5	0.20
41	31.538	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	356.5	0.19
42	31.668	(Z)-Trimethyl(tetradec-11-en-1-yloxy) silane	226.4	0.48
43	31.800	9-Methyl-Z, Z-10,12-hexadecadien-1-ol acetate	294.5	0.91
44	32.273	Oleic Acid	282.4	7.16
45	32.394	Tricosane	324.6	5.74
46	32.577	1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-yl)-, 1,1-dioxide	264.3	2.87
47	32.764	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	312.4	1.80
48	33.000	cis-11-Hexadecenal	238.4	4.94

Table 2 (cont): GC-MS Analysis of Ethyl acetate fraction of *O. Longinoda*

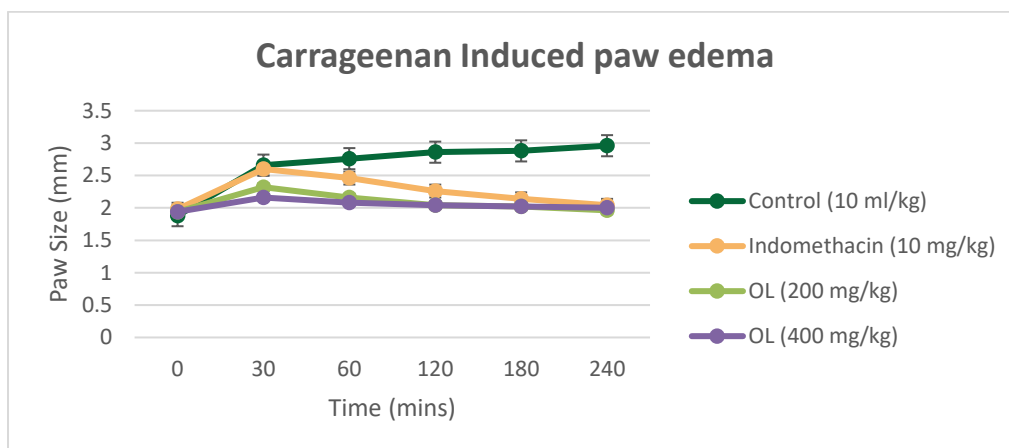
Peak No.	Retention Time (min)	Area Percent (%)	Compound	Retention Time (min)	Area Percent (%)
49	33.960	10.84	Oleic Acid	282.4	10.84
50	34.126	4.81	Oleic Acid	282.4	4.81
51	35.320	5.25	Oleic Acid	282.4	5.25
52	35.373	0.87	Oleic Acid	282.4	0.87
53	35.474	2.95	Oleic Acid	282.4	2.95

In Table 2, the major components detected from the methanol fraction of *O. longinoda* were Carvone (Retention time (Rt): area percent: 13.547: 21.12%) a terpenoid: oleic acid (33.960, 10.84%), oleic acid (32.273:7.16%), oleic acid (35.320: 5.25%), Tricosane (32.394, 5.74%) an hydrocarbon, cis-11-hexadecenal (33.000: 4.94%) an aldehyde and hexadecanoic acid, methyl ester (29.548:4.65%). Carvone is a terpenoid found naturally in many essential oils. It is a monoterpene ketone (2-methyl-5-(1-méthylethényl)-2-cyclohexen-1-one) ($C_{10}H_{14}O$) that has a variety of pharmacological properties, including antibacterial, antifungal, antiparasitic, antineuraminidase, antioxidant, anti-inflammatory, and anticancer qualities (Bouyahya et al., 2017). It is an allergen that can be used as an anti-fungal agent, which has been investigated against various fungi strains: *Candida* spp., mycotoxigenic fungi and dermatophytes (Bouyahya et al. 2017). Oleic acid is used in medicines as an excipient and in aerosol goods as an emulsifying or solubilizing agent. The acid may potentially be responsible for olive oil's hypotensive (blood pressure-lowering) properties (Frag and Gad, 2022). This acid has anti-proliferative activity, which includes suppression of migration and proliferation of breast cancer cells, as well stimulation of tumor

suppressor genes (Frag and Gad, 2022). The detection of methyl decanoate in peak 27 (Table 2) also corroborates the findings of researchers who isolated methyl decanoate from petroleum ether fraction of adult workers of weaver ants while the alcoholic compound, 13-octadecadienol (peak5, Table 2) have also been suggested as members of trail pheromones of the ant *Leptogenys peuqueti* (Igwe and Eze, 2015). The anti-inflammatory properties of oleic acid (OA), has been established in some studies (Frag and Grad 2022). Oleic acid has an anti-proliferative impact, reducing breast cancer cell migration and proliferation while also activating tumor suppressor genes (Frag and Gad 2022). Several investigations have discovered that oleic acid suppresses cell development in a variety of tumor cell types (Carrillo et al., 2012).

3.3 Carrageenan-induced paw edema

OL significantly decreased paw volumes from the 2nd hour to the 4th hour at all tested doses of the extract demonstrating paw size reduction. OL (both 200 and 400 mg/kg) outperformed indomethacin particularly at the 4th hour as seen in Figure 3.

**Figure 3:** Effect of OL on carrageenan induced paw edema

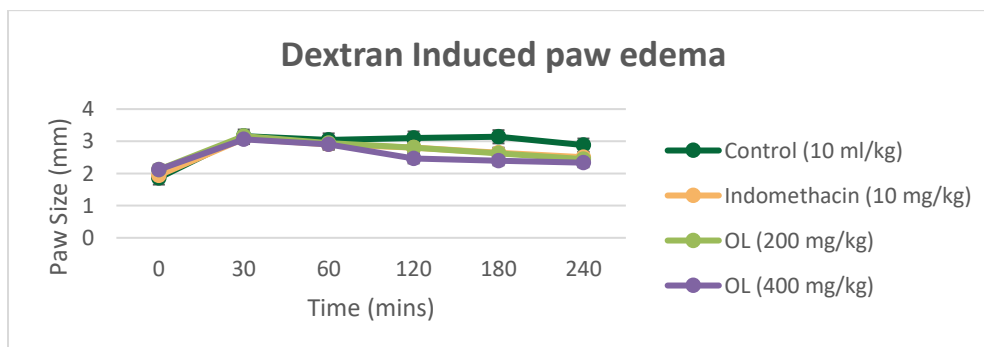
Values are expressed in mean \pm SEM, n=5 per group. Significance values * $P < 0.001$ significantly different from the control. Control animals received tween 80 in distilled water.

The inhibitory impact was observed in the first and second phases and lasted until the fourth hour (the maintenance phase of inflammation). Inhibition of the first phase shows that the extract contains antihistamine action, which might reduce micro vascular leakage caused by carrageenan (Kuriyama et al., 2010). Histamine activates artery endothelial cells to improve vascular permeability, resulting in an outpouring of cells and fluid (Kuriyama et al., 2010). Since the carrageenan inflammatory model essentially mimics the action of prostaglandins, reduction of the second phase implies a probable inhibition of cyclooxygenase production (Kuriyama et al., 2010). The impact of the extract on the maintenance phase suggests that it inhibited the release of bradykinin and/or promoted

vascular permeability. The effect is comparable to that caused by anti-inflammatory medications such as indomethacin, whose mechanism of action is based on the suppression of the cyclooxygenase enzyme, which catalyzes the production of cyclic endoperoxides, which are necessary for the generation of prostaglandins (Mori and Abe, 2022)

3.4 Effect of OL on dextran induced paw edema

Treatment with OL significantly reduced the paw sizes from the 1st to the 4th hour compared to the control group. The effect of OL was seen to be significant from the 2nd to the 4th hour especially in the 400 mg/kg dosage. The significance of OL's effect was generally not dose-dependent compared to the control. This can be seen in Figure 4. The effect of OL was better than indomethacin especially at the 2nd to the 4th hour, particularly for the 400 mg/kg dosage.

**Figure 4:** Effect of OL on Dextran induced paw edema

Values are expressed in mean \pm SEM, n=5 per group. Significance values *P<0.001 significantly different from the control. Control animals received tween 80 in distilled water.

The dextran-induced paw edema model was utilized to investigate OLs' immediate anti-inflammatory activity. OL dramatically decreased paw volumes as an indication of dextran-induced edema. Dextran, being a strong osmotic agent, causes anaphylactic reactions. The inflammatory reaction induced by dextran, like other inflammatory agents, is divided into two parts. Extravasation and edema development as a result of histamine generate a considerable increase in vascular permeability and blood flow to the inflammatory site during the early phase (0-1 h). When

compared to the control (tween 80), the oil fraction of OL prevented paw edema at dosages of 200, and 400mg/kg, with their impact being considerably obvious from the second hour up to the fourth hour, particularly for the 400mg/kg dosage and this results showed a significant difference with a p-value of 0.001.

3.5 Effect of OL on Formaldehyde induced paw edema

Figure 5 shows the effect of OL on formaldehyde induced paw edema. At the 400 mg/kg dose, OL had significant effect (p<0.05) in comparison with the control.

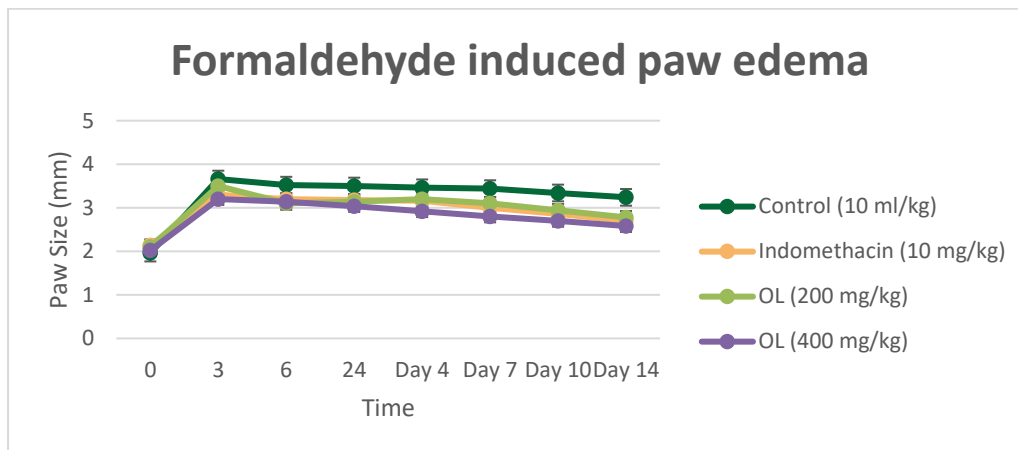


Figure 5: Effect of OL on Formaldehyde induced paw edema

Values are expressed in mean \pm SEM, n=5 per group. Significance values *P<0.05 significantly different from the control. Control animals received tween 80 in distilled water.

The suppression of edema generated by formaldehyde in rats is one of the most appropriate test protocols for screening anti-arthritis (chronic type of inflammation) and anti-inflammatory drugs (Chen et al., 2020). Inflammation caused by formaldehyde is a model used to assess the anti-proliferative action of a potential drug and in this study; the oil fraction had a slight significant inhibitory effect at the 400mg/kg dosage (p-value of 0.05) (Chen et al., 2020). The findings reaffirm a probable influence of the extract on formaldehyde-induced cell damage.

One of the key biochemical processes throughout the inflammatory process is the induction of formalin, which induces alterations in connective tissue metabolism. These changes are reflected in the relative composition of numerous connective tissue elements such as mucopolysaccharides, glycoprotein, hexosamine, and hydroxy proline, sialic acid. As a result, hexosamine and hydroxyproline levels were shown to be greater in formalin-induced rats. OL pretreatment decreased the accumulation of hydroxyproline and hexosamine in edematous tissue of formalin-induced rats (Vettore et al., 2021).

3.6 Acute toxicity:

The animals treated with the extract showed no sign of toxicity which included; alertness, touch response, tremor, pinna reflex and writing reflex. Control animals received distilled water. (Table 3)

GROUPS	Dose Mg/kg	Sign of Toxicity	Mortality (%)
Control	0.2 ml	Nil	0
Extract	10	Nil	0
Extract	100	Nil	0
Extract	1000	Nil	0
Extract	1600	Nil	0
Extract	2900	Nil	0
Extract	5000	Nil	0

The oral acute toxicity test of the OL oil fraction was performed using the modified method and the results showed that the animals at doses of 10, 100, and 1000mg/kg for the first phase, as well as 1600, 2900, and 5000mg/kg for the second phase, did not show any signs of toxicity (alertness, touch response, tremor, pinna reflex, writing reflex, etc.) and death (mortality) during the observation period (Ogbeide et al., 2022). The findings ruled out any harmful effects of the extract.

4. CONCLUSION

This study revealed that the oil fraction of methanol extract of OL had an anti-inflammatory effect in rats with the three models. This results thus, compares favorably with the conventional medication, indomethacin. The findings indicated a beneficial effect on both acute and sub-chronic inflammation, which might be attributed to the control of inflammatory mediators and the suppression of inflammation gene expression. The presence of flavonoids as a bioactive constituents detected in the oil fraction might explain the observed anti-inflammatory action. More research is needed to investigate the mechanisms of anti-inflammation, as well as adequate characterization of the active compounds in the oil fraction.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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