



Arch. Bas. App. Med.11 (2023):62-68

www.archivesbamui.com

www.ojshostng.com/index.php/abam

Research Article

Methanol leaf extract of *Terminalia superba* Engl & Diels (Combretaceae) mitigates chemically induced nociceptive and inflammatory effects in animal models

Ani, N.I.¹, Nworu, C.S.², *Okolo, K.O.¹, Apeh, O.K.¹. and Ugwu, E.C.¹

¹Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology, Enugu, Nigeria

²Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu, 410001, Nigeria

Accepted: 28 July, 2023

Abstract

The effect of methanol extract of *T. superba* (METs) leaves on nociception and inflammation were investigated in this study. Folk medicine has reported the use of *T. superba* in the management of swelling, but scientific evidence of its usage is limited. This study aims to study the nociceptive and anti-inflammatory properties of *Terminalia superba*. Fifty Sprague Dawley rats of either sex were split into two major groups with five sub-groups (n=5). Each of the major groups was used for formalin and cotton pellet granuloma models. Also, 50 mice divided into 2 major groups of 25 with 5 groups were used for analgesic writhing and thermal response tail-flick tests in mice. 100, 200 and 400 mg/kg were used while diclofenac (10 mg/kg) and morphine (10 mg/kg) served as standard drugs for anti-inflammatory and analgesic investigations respectively. METs at 200 and 400 mg/kg ($p < 0.05$) inhibited the inflammatory action of the formalin and cotton pellet granuloma significantly, in a manner that is dose-dependent. METs also significantly ($p < 0.05$) reduced writhing and thermal response. Based on these results, METS have both anti-inflammatory and anti-nociceptive potentials which could explain its folkloric use in the management of swelling.

Key Words: *Terminalia superba*, inflammation, nociception, granuloma, pain threshold

INTRODUCTION

Inflammatory responses produced and controlled by pro-inflammatory mediators may give rise to a complicated cascade of activities if not well managed (Patil *et al.*, 2019). These highly controlled and regulated protective responses, which help eliminate the initial cause of cell injury and begin repair processes, are termed inflammation (Gautam & Jachak, 2009; Singh & Sharma, 2013). An excessive cascade of inflammatory actions due to mediators derived either from leucocytes, damaged tissues or harmful stimuli such as irritants and pathogens, have led to persistent ailments such as cancer, rheumatoid arthritis, asthma, just to mention a few (Patil *et al.*, 2019). Certainly, where and whenever there is inflammatory disease, the most common patient complaint is nociception and fever. Most of the mediators of inflammation such as neuropeptides, kinins, cytokines and arachidonic acid metabolites which initiate inflammation also mediate nociception (Nworu & Akah, 2015; Rauf *et al.*, 2017). Inhibition of inflammatory pathways will not only curb inflammation but will also arrest pain. For instance, prostaglandin E2 (PGE2) sensitizes nerve ending to the actions of bradykinin, histamine and other autacoids released during an inflammatory process. So, the inhibition of cyclooxygenase 1 and 2 enzymes will not only manage inflammation (Nworu & Akah, 2015; Eze *et al.*, 2019) but will

also cut off the production of (PGE2) which is implicated in nociceptive nerves sensitization.

The drugs which are most commonly used to manage or treat episodes of inflammation and pain are the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) like aspirin and diclofenac. However, the unwanted side effects expressed by this drug group on the gastrointestinal tract (gastritis/ulcers), immune system (muscular degenerations), heart (hypertension, cardiovascular infarction), hair and blood cell counts have placed strict restrictions and limitations on its clinical use (Fan *et al.*, 2014). Consequently, there has arisen a need for products which will alleviate both pain and inflammation which are more natural, whose side effects potentials are few and which are not deleterious to the human system.

In recent times, medicinal plants are perceived to be the most well-known type of alternative medicine (Mussarat *et al.*, 2021). In Nigeria which is categorized as a developing country, alternative medicines in the form of natural or herbal prescriptions are frequently used in the treatment and management of diverse illnesses and this indemnifies the irregularities encountered with orthodox pharmacotherapy. These natural medicinal plants are widely available in Nigeria and their herbal preparation is usually more affordable compared to those of orthodox medicine (Osifo *et al.*, 2022; Ani *et al.*, 2023).

The natural medicinal plant of focus for this study is the *Terminalia superba*. It is a huge tree from the family, Combretaceae and is home-grown in tropical west Africa. It's native names include korina (US), the superb terminalia, frake (Africa), ofram (Ghana), limba or afara (UK), African limba wood (Fifamè *et al.*, 2018).

Folkloric claims have described the stem bark as astringent, anodyne and expectorant. It is used in traditional medicines as decoctions and macerations to treat lacerations, sores, dysentery, malaria, hemorrhoids, diarrhea, and gingivitis, inflammations of the bronchi, aphthae, vomiting, swellings and disorders of the ovaries. Some pharmacological works have reported the ability of stem bark to heal wounds (Kwete *et al.*, 2010; Ani *et al.*, 2023). This study steered at ascertaining the anti-inflammatory and analgesic potentials of the leaves of *T. superba*.

MATERIALS AND METHODS

Plant collection, identification and extraction: The *T. superba* leaves were gotten from Enugu state, Nigeria, precisely at Nsukka. A specimen voucher (InterCEDD/203) was placed with the International Center for Drug Research and Development (InterCEDD), Nsukka, Enugu State, Nigeria, after a botanist affiliated with the Bioresources Development and Conservation Program (BDPC) identified and verified the plant component utilized. The collection was done in the early hours of the day to preserve the plant leaves' phytochemical content. It was air-dried in shade for a few days and ground to a coarse powder. The method of extraction employed was the cold maceration process. The coarsely ground powdered leaves were macerated using methanol in a ratio of 1:5. A total of 1100g of the sample was macerated in 5500ml (5.5L) of methanol for three days in large amber bottles with 8 hourly agitations. Using the Whatman filter paper, the non-exhaustive extraction material was filtered to gain the methanol extract of *Terminalia superba* (METs). The extract was reduced to one tenth of its volume using a rotary evaporator and to dryness using a water bath 40°C.

Experimental Animals: The laboratory animal facility at the Department of Pharmacology and Toxicology at the University of Nigeria's Nsukka provided adult Swiss mice and Sprague dawley rats of any gender, whose weight ranges are 23–27g and 180–200g, respectively. In the animal house facility, the animals were divided into distinct metal cages and freely given admittance to potable water and pellets for rodents as food. A comfortable room temperature, a cycle of light and dark times of 12 hours each, and a well-ventilated room was provided for the animals to ensure their utmost acclimatization. Conduction of animal experiments followed the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub. No. 85-23, revised 1985) and in agreement with the Ethics Committee of the University on laboratory animals handling (PAHRM/22/34).

Acute toxicity test: There was an assessment of the acute toxicity and mean lethal dose (LD50) of METs in mice using the techniques explained by Lorke (1983). First, three groups of nine mice (n=3) were created, and each group received oral administration of 10, 100, or 1000 mg/kg of METs prepared in 3% tween 80. After 24 hours, each group was assessed for toxicological manifestations like behavioral changes, neural

and autonomic toxicities, feeding pattern changes, etc. After 24 hours, no deaths were noted. In the second stage of the investigation, fresh mice were divided into four groups (n=1) and given 1600, 2900, 3600, or 5000 mg/kg of METs. After 24 hours, the mice were also observed for toxicological manifestations like behavioral changes, neural and autonomic toxicities, feeding pattern changes, etc. or death.

Phytochemical analysis: The methanol extract of *T. superba* was subjected to a phytochemical screening in consonance with typical procedures (Evans, 2009; Harbourne, 1998).

Acetic Acid-Induced Writhing Test: Writhing test using the acetic acid-induced model was done following the method reported by (Tsai *et al.*, 2015). Twenty-five (25) mice of any sex were blindly split into 5 different groupings (n=5) and labelled A to E. Each group represents a particular dose of the extract or the controls. Groups A and B represent control (5 mL/kg distilled water) and standard drug (10mg/kg morphine) respectively, while groups C to E represent the 100mg/kg extract group, 200mg/kg extract group, and 400mg/kg extract group respectively. Acetic acid solution (1% acetic acid of 10 mL/kg body weight) was given to each mouse intraperitoneally exactly thirty (30) minutes after treatment with either the standard drug or the methanol crude extract per oral. Separated in transparent cages, the animals were individually observed for writhes. A writhe was considered to be a lone muscular contraction of the abdomen with stretching of the hind limb. Observation of each animal shortly after the acetic acid was administered for a period of 30 minute. Percentage inhibition of writhing was estimated by the following formula:

$$\text{Percentage inhibition of writhing} = [1 - W_t/W_c] \times 100.$$

Where W_c and W_t represent the mean number of the writhing of the control group and test group, respectively.

Thermal Response Investigation Using Tail Flick Method: The tail-flick thermal response method proposed by D'Amour and Smith (1941) and modified by Singh *et al.*, 2018 was used to assess the analgesic (anti-nociception) effects of the METs. Each rat had its distal end of the tail (approximately 5 cm) immersed in warm water maintained at 50°C. The reaction time (RT) (in seconds), which was calculated as the average of two readings, is the length of time it takes each rat to flick its tail in response to pain. The RT (0 min) was noted before the treatments were administered and at 30, 60 and 90min after the administration of the treatments. In order to avoid any injury to the tissues of the tail, the maximum RT was fixed at 15 secs. Maximum analgesia was considered to be any reading that goes beyond 15 seconds. The mean RT obtained before the plant extract or drug was administered was considered the baseline (BL). Anti-nociception was quantified as the percent maximal possible analgesia (MPA) and calculation was done with the following formula:

$$\% \text{MPA} =$$

$$\frac{\text{Reaction time for treatment} - \text{reaction time for saline}}{15 \text{ secs} - \text{reaction time for saline}} \times 100$$

Rats were randomly distributed into five groups (n=5). As a negative control, batch 1 was given 10 mL/kg of distilled water. *Terminalia superba* leaf methanol extract was administered to batches 2, 3, and 4 at doses of 100, 200, and 400 mg/kg, respectively. The final group acted as the positive control and received 10 mg/kg of morphine sulfate. Treatments were all administered intraperitoneally.

Cotton pellet-induced granuloma in rats: In accordance with the method described by Rathi *et al.*, (2004) cotton pellets were inserted into the animals. Five groups of 25 rats each were created at random (n = 5). A 5 mL volume of distilled water was given to Group I (the control group). Groups III, IV, and V each received an oral treatment dose of 100, 200, and 400 mg/kg METs, respectively, while Group II, the positive control, received diclofenac potassium 10 mg/kg. Pentobarbitone was used to anesthetize the animals, and after a single incision that was later stitched up with broken sutures were made, with each rat receiving a subcutaneous injection of a sterile cotton pellet (0.02g) made of bleached cotton and saturated with normal saline. Individual animals were kept separately in a metal cage to avert animals licking or attacking each other's wounds and stringent sterile measures were maintained all through the periods or duration of the study. Recovery time from the anaesthesia and time for inflammatory events to commence was allowed for the animals before they started receiving oral treatment of the methanol extract of *Terminalia superba* or diclofenac once every day for 14 days. The calculation of the final dry weight after subtracting the original weight of cotton pellets was done and the result was taken as a measure of granuloma tissue formation (Su *et al.*, 2011). Wet weight of cotton pellet = Weight of the cotton pellet (wet) - Original Weight of the cotton pellet. The dry weight of cotton pellet = Weight of the cotton pellet (dry) - Original Weight of the cotton pellet. Quantification of the rise in both the wet and dry weights of the formed granuloma tissue was in relation to the control (vehicle).

$$\% \text{ edema inhibition} = \frac{W_c - W_t}{W_c} \times 100$$

Where W_c = difference in pellet weight of the control group and W_d = difference in pellet weight of the drug-treated group.

Formalin-Induced Mice Paw edema: Anti-inflammatory investigation was conducted on formalin-induced inflammatory model using the method described by Turner (Turner, 2011). Animals were indiscriminately split into 5 groups (n=5). The paw size was taken at zero time, before the injection of 0.1mL of freshly prepared 2% formalin in the right hind paw, sub-plantar. The extract at different doses of 100, 200, and 400 mg/kg or diclofenac 10 mg/kg and distilled water (5mL/kg.) were given per oral immediately after the induction of inflammation without taking paw size. Daily variations in paw edema were computed using a volumeter (an instrument for measuring volumes) prior to subsequent treatments. The extracts and control treatments were carried out for 7 consecutive days.

$$\% \text{ edema inhibition} = 100 \times (1 - V_t/V_c)$$

Where V_c represents mean oedema in control and V_t means oedema in the group treated with standard/extract.

Statistical analysis: Collected data was analyzed by one-way analysis of variance (ANOVA) and subsequently by Dunnett's multiple comparisons post-hoc test using Graph pad Prism version 5.0. The values represent mean and their standard error of the mean (SEM). $p < 0.05$ was considered to be statistically significant.

RESULTS

Results of phytochemical analysis of METs : Table 1 shows the phytoconstituents contained in *T. superba* leaf extract as observed in the qualitative test. There was abundant presence of tannins, alkaloids, flavonoids, saponins, phenols, quinones, amino acids and proteins.

Table 1:
Phytoconstituents detected in METs.

Constituents	Relative abundance
Alkaloids	+++
Flavonoids	+++
Saponins	+++
Steroids	-
Phytosterols	-
Phenols	+++
Glycosides	-
Quinone	+++
Terpenoids	-
Tannins	+++
Coumarins	+++
Carbohydrates	+
Proteins	+++
amino acids	+++

-Not present, +present in small concentration, ++present in moderately high concentration, +++ abundantly present.

Table 2:
Effects of METs on acetic acid-induced writhing test in mice

Groups	Doses	Number of writhes	% Writhes inhibition
Distilled water	5 ml/kg	13.8 ± 1.48	-
Morphine	10 mg/kg	4.6 ± 0.89***	66.7
METs	100 mg/kg	7.8 ± 0.84**	43.5
METs	200 mg/kg	5.4 ± 1.14**δ	60.9
METs	400 mg/kg	4.2 ± 1.30**δ	69.6

Values are presented as Mean ± SEM; each group has 5 animals. One-way Analysis of Variance (ANOVA) was used to estimate the results, and then Dunnett's post hoc test was performed. ** and ***: $p < 0.01$ and 0.001 respectively compared to 5 ml/kg distilled water (vehicle control); δ $P < 0.05$ compared to Morphine (standard drug)

Effects of METs on acetic acid-induced writhing test in mice: Treatment with METs 100, 200 and 400 mg/kg, $p < 0.05$ produced significant ($p < 0.05$) inhibition of the abdominal contractions in acetic acid-induced animals as compared to the

negative control. METs at 100, 200 mg/kg produced inhibition less than that of morphine while 400 mg/kg showed a significant inhibitory difference ($p < 0.05$) better than morphine.

Effects of METs on the tail-flick thermal response test in mice: When compared to distilled water 10 ml/kg, METs at all doses: 100, 200, and 400 mg/kg, p.o., was significant ($p < 0.05$). 400 mg/kg of METs at 60 and 90 minutes considerably ($p < 0.05$) increased the thermal response threshold in mice, which is comparable to morphine.

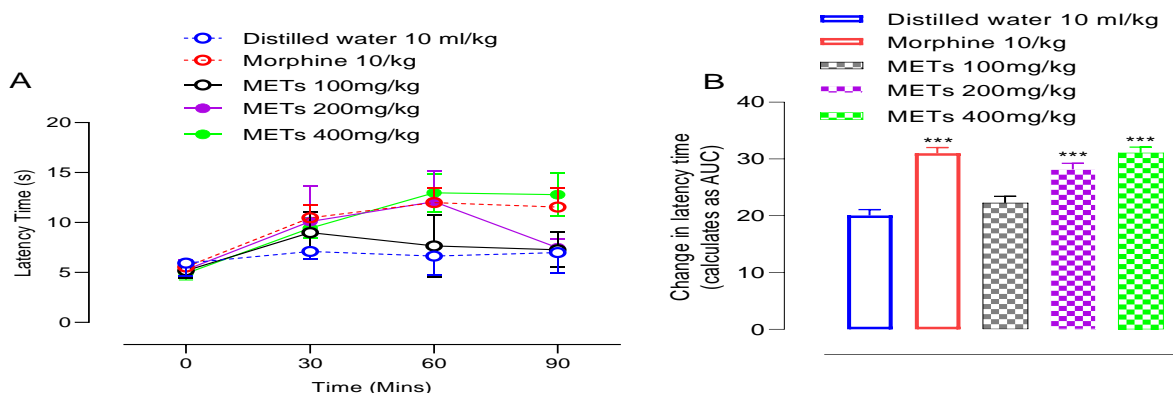


Figure 1: Effect of METs on nociceptive in animals exposed to tail-flick test. (a) Lapsed time against change in time and (b) change in lapsed time calculated as AUC. Values are presented as Mean±SD; each group has 5 animals. One-way Analysis of Variance (ANOVA) was used to estimate the results, and then Dunnett's post hoc test was performed. ***: $p < 0.001$ compared to the negative control.

Effects of METs on cotton pellet granuloma-induced inflammation in rats: METs at the dose of 400 mg/kg significantly ($p < 0.05$) inhibited the wet and dry weights of the cotton pellet at 27.43 and 53.13% respectively just like the affect produced by diclofenac 10 mg/kg for the wet weight (25.14%) and dry weight (35.94%). The dry weight percentage inhibition of 200 mg/kg METs is also significant ($p < 0.05$) and comparable to diclofenac.

Effects of METs on Formalin-induced oedema: All the doses of METs remarkably ($p < 0.05$) inhibited the oedema induced by the administration of formalin in rats. A graded dose-response was observed as an increase in dose showed increased percentage inhibition at all times within the 7 days of treatment with METs

Table 3: Effects of METs on cotton pellet granuloma-induced inflammation in rats

Treatment	Dose	Wet weight (g)		Dry weight (g)	
		Mean ± SEM	% inhibition	Mean ± SEM	% Inhibition
METs	100 mg/kg	1.63 ± 0.14	05.71	0.59 ± 0.07	7.81
METs	200 mg/kg	1.58 ± 0.07	09.71	0.40 ± 0.04**	37.50
METs	400 mg/kg	1.27 ± 0.06**	27.43	0.30 ± 0.05***	53.13
Diclofenac	10 mg/kg	1.31 ± 0.12**	25.14	0.41 ± 0.02**	35.94
Distilled Water	5 ml/kg	1.75 ± 0.06		0.64 ± 0.06	

Values are presented as Mean ± SEM; each group has 5 animals. One-way Analysis of Variance (ANOVA) was used to estimate the results, and then Dunnett's post hoc test was performed. **, *** $p < 0.01$ and 0.001 respectively in comparison to the standard reference and the negative control.

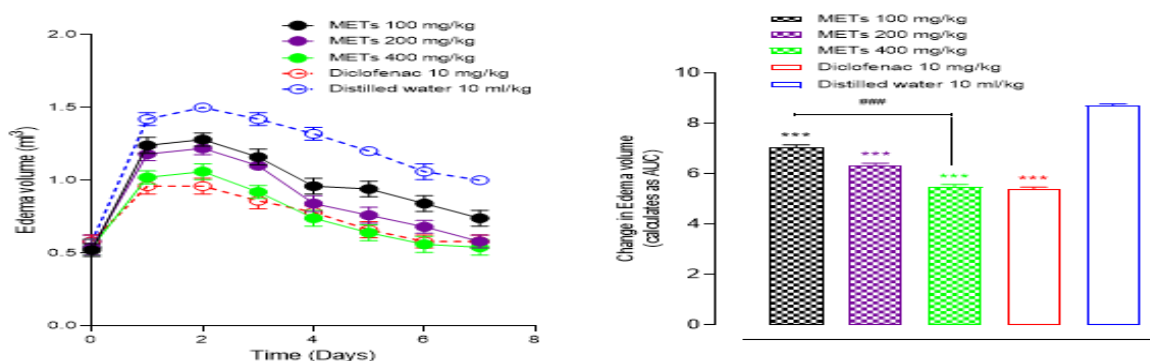


Figure 2: Effect of extract of formalin-induced paw oedema represented as (a) edema volume against time in days, and (b) change in edema volume calculated as AUC.

Values described as Mean \pm SD; n=5 animals per group. One-way Analysis of Variance (ANOVA) was used to estimate the results, and then Dunnett's post hoc test was performed. *, ** and ***: P < 0.05, 0.01 and 0.001 respectively in relation to 10 ml/kg distilled water (vehicle control); ### P<0.001 compared to Diclofenac Na (reference standard)

DISCUSSION

The medical, social and economic burden of chronic inflammatory diseases and pain of various origins still persist despite achievements recorded in their pharmacotherapy. Research in these areas is still ongoing to improve the treatment and management of these unresolved medical conditions. This research investigated the efficacy of methanol leaf extract of *T. superba* (METs) on nociception and inflammation. The methanol crude extract of *T. superba* leaves up to the dose of 5000 mg/kg through oral administration caused no observable toxicological signs as there are no changes in feeding patterns, neurological or behavioral abnormalities. There was also no death recorded in both phases of the acute toxicity conducted as postulated by Lorke (1983). The result obtained suggests that the toxic dose is above 5000 mg/kg and METs appeared relatively safe for use.

Drugs or agents which act on both the central and peripheral nervous systems to selectively relieve pain without a remarkable alteration in consciousness are called analgesics (Moore et al., 2013). Usually, a centrally acting analgesic raises the pain threshold and alters physiological responses to pain while the peripherally acting analgesics act at the chemoreceptor pain sites to inhibit the generation of impulses at these sites (Fan et al., 2014; Stollenwerk et al., 2018). In addition, persuading proof has indicated that PGE-2 can cause stomach tightening (Nesa et al., 2018), and it is for the most part liable for causing provocative inflammatory pain.

The tail-flick test, a pain-state model that uses thermal stimuli, was used to assess the anti-nociceptive capacity of mice. The tail-flick technique causes a spinal reaction in response to a nociception stimulus, instantiating a centrally mediated anti-nociception with a general focus on variations beyond the spinal cord level whereas, the acetic acid when administered intraperitoneally, produces both central and peripheral nociception caused by the release of endogenous pro-inflammatory mediators (Costa et al., 2019). METs showed a significant decrease in writhing and abdominal stretching prompted because of acetic acid, and in the flicking of the tail induced by a thermal stimulus in mice indicating the presence of analgesic principles with the ability to attenuate both inflammatory and thermal-mediated pain. The results obtained in the tail flick as well as acetic-acid investigations for the analgesic property of METs may be related to the stoppage of the endogenous substances release such as the cytokines and arachidonic acid metabolites (Moni et al., 2021) which play important roles in the central pain mechanism at the brain and spinal cords (Eze et al., 2019). This result agreed with a work done by Pokuri et al., 2016, where administration of specie of terminalia, known as *T. chebula* to health volunteers in a randomized, double blind, placebo controlled and cross over study increased the mean percentage change for pain threshold force and time by 12.8, and 21% respectively. Furthermore, Jami et al., 2014 showed that there is a significant increase in the mean reaction time of animals to heat stimuli in a hot plate model at both 250 and 500 mg/kg body weight of *T. chebula* leaf extract. These corroborated out study as the doses of 100, 200 and 400 mg/kg showed improvement in reaction time to

tail flick. This analgesic property could be attributed to the presence of compounds like ellagic acid, flavogallonic acid, quercetin that had been identified in another specie of terminalia, known as *T. macroptera* (Haïdara et al., 2020).

Acute inflammation which is usually mediated by the immune system actuation is often described as the initial stage of inflammation and constitutes the body's response to injury (Bagad et al., 2013). It persists for some time and may be of beneficial to the host. But when it lingers for a long period of time, chronic inflammation sets in and will usually predispose the host to various forms of chronic illnesses and disorders (Reuter et al., 2010; Abdelgadir & Van Staden, 2013). Whenever chronic inflammations occur, it shows that the acute response is not sufficient to curb the pro-inflammatory agents. At this point, neutrophils infiltrates, fibroblasts proliferate and there is fluid permeability and exudation (Navegantes et al., 2017). There is the emergence of these prolific thriving cells which exist in the form of granuloma or can spread more into the system (Reuter et al., 2010; Eze et al., 2019).

The cotton pellet granuloma model was helpful to ascertain the exudative, transudate and proliferative dispositions of long-term inflammations. While the transudate phase results to an appreciation in the wet weight of the cotton pellet, the inflammatory responses to the embedded cotton pellet at about the 3rd to the 6th day of the experiment cause granuloma formation (Okoli et al., 2006; Bagad et al., 2013) revealing the levels of cell proliferation in response to inflammatory stimuli. In this study, the wet and dry weight of METs showed a significant decrease in both transudation and proliferation respectively. This may be attributed to the potential of METs being able to suppress the activities of pro-inflammatory molecules like interleukins (IL); IL-6, IL-1, IL-2 (Ma et al., 2011), TNF- α , inflammatory arachidonic acid (AA) metabolites (Glass et al., 2010) and enzymes which are catalytic to AA metabolic pathway such as phospholipase A, cyclooxygenases (I and II) and lipoxygenase enzymes (Li et al., 2021). This is in agreement with the works (Fan et al., 2004; Fahmy et al., 2017; Gupta et al., 2021), where other species of terminalia exhibited anti-inflammatory properties that had been attributed to phytochemicals present in the plant. Formalin-induced edema model helps to investigate the activity of a test drug on long lasting inflammation. Its ability to produce edema happens in 2 phases. The first phase credits its assertions to the discharge of serotonin (5-HT) in addition to histamine and kinins (Navegantes et al., 2017) while the edema formed at the second phase is majorly mediated by prostaglandins (Mbiantcha et al., 2017). The suppression of inflammatory cytokines and mediators as well as the down regulation of these molecules may be responsible for METs' anti-inflammatory effects. The anti-inflammatory results agreed with similar works (Abiodun et al., 2016; Chauhan et al., 2018; Rodrigues De Araújo et al., 2019). According to Lucas et al., (2006), anti-inflammatory activities of medicinal products do not only halt the effects of cyclooxygenases, but also those of lipoxygenases and inducible nitric oxide synthases (Nworu & Akah, 2015). Using both in vitro and in vivo techniques, (Abiodun et al., 2016) and (Vijayalakshmi et al., 2023) proved the efficacy of terminalia species in combating inflammatory mediators. Infact, *T. mollis* root bark's anti-inflammatory property is believed to be mediated

via the presence of 12 – myristate – 13 – acetate containment of neutrophils infiltration (Muganga *et al.*, 2020). Also, the detection of the presence of flavonoids, saponin, tannin, phenols, coumarin, alkaloids as the phytochemical secondary metabolites may be useful for the activity of METs which is in consonance to their anti-inflammatory (Ambriz-pérez *et al.*, 2016; Elisha *et al.*, 2016) and analgesic capacities and potentials. A number of flavonoids have been shown to create pain-relieving activity through the concealment of PGE-2 synthesis. A phytochemical profiling work done by (Shahzad *et al.*, 2022) showed that tannins and flavonoids may be the major active principles exhibiting both anti-inflammatory and analgesic properties, and identified gallic acid, quercetin, and ellagic acid as the main compounds.

CONCLUSIONS

According to the findings of this investigation, *T. superba*'s methanol extract exhibits both analgesic and anti-inflammatory activities because of its ability to significantly curb both pains and swelling in in-vivo models. Due to the promising results reported in this study, further studies are advocated to be carried out on this plant to ascertain the mechanism of the activities of *T. superba*'s methanol extract.

REFERENCES

- Abdelgadir, H. A., and J. Van Staden. 2013. Ethnobotany, ethnopharmacology and toxicity of *Jatropha curcas* L. (Euphorbiaceae): A review. *South African Journal of Botany*, 88: 204–218.
- Ambriz-pérez, D. L., N., Leyva-lópez, E. P Gutierrez-grijalva, and J. B. Heredia. 2016. Phenolic compounds: Natural alternative in inflammation treatment: A Review. *Cogent Food & Agriculture*, 41(1): 1-14.
- Ani, N. I., K. O. Okolo, and R. O. Offiah. 2023. Evaluation of antibacterial, antioxidant, and anti-inflammatory properties of GC/MS characterized methanol leaf extract of *Terminalia superba* (Combretaceae, Engl. & Diels). *Future Journal of Pharmaceutical Sciences*, 9(3): 1-14.
- Bagad, A. S., J. A. Joseph, N. Bhaskaran, and A. Agarwal. 2013. Comparative Evaluation of Anti-Inflammatory Activity of Curcuminoids, Turmerones, and Aqueous Extract of *Curcuma longa*. *Adv Pharmacol Sci*. 2013:805756: 1-7.
- Costa, F. V., L. V. Rosa, V. A. Quadros, A. R. Santos, A. V. Kalueff, and D. B. Rosemberg. 2019. Understanding nociception-related phenotypes in adult zebrafish: Behavioral and pharmacological characterization using a new acetic acid model. *Behavioural Brain Research*, 359, 570-578.
- D'Amour, F. E., and D. L. Smith. 1941. A method for determining loss of pain sensation. *J Pharmacol Exp Ther*, 72(1): 74-9.
- Elisha, I. L., J. P. Dzoyem, L. J. McGaw, F. S. Botha, and J. N. Eloff. 2016. The anti-arthritic, anti-inflammatory, antioxidant activity and relationships with total phenolics and total flavonoids of nine South African plants used traditionally to treat arthritis. *BMC Complementary and Alternative Medicine*, 16(1): 1–10. <https://doi.org/10.1186/s12906-016-1301-z>
- Evans, W.C., 2002. Trease and evans. Pharmacognosy, 9th Edition published by Saunders Elsevier, 553 pp.
- Eze, F.I., Uzor, P.F., Ikechukwu, P., Obi, B.C. and Osadebe, P.O. 2019. In vitro and In vivo Models for Anti-inflammation: *An Evaluative. enzyme*, 1(1), p.278.
- Fahmy NM, Al-Sayed E, Abdel-Daim MM, Singab AN. 2017. Anti-inflammatory and analgesic activities of terminalia muelleri benth.(combretaceae). *Drug development research*, 78(3-4), 146-154.
- Fan, S., N. A. Ali, and D. F. Basri. 2014. Evaluation of Analgesic Activity of the Methanol Extract from the Galls of *Quercus infectoria* (Olivier) in Rats. *Evidence-Based Complementary and Alternative Medicine*. 976764: 1-6.
- Fan YM, Xu LZ, Gao J, Wang Y, Tang XH, Zhao XN, *et al.* 2004. Phytochemical and antiinflammatory studies on *Terminalia catappa*. *Fitoterapia*, 75(3), 253-260. doi:<https://doi.org/10.1016/j.fitote.2003.11.007>
- Fifamè, K., E. Elvire, and A. D. Casimir. 2018. Antioxidant and Antibacterial Activities of *Terminalia superba* Engl. and Diels (Combretaceae) Bark Extracts. *Int. J. Curr. Microbiol. Appl. Sci*, 7, pp.2836-2846.
- Gautam, R., and S. M. Jachak. 2009. Recent Developments in Anti- Inflammatory Natural Products. *Med Res Rev*. 29(5):767-820 <https://doi.org/10.1002/med>
- Glass, C. K., K. Saijo, B. Winner, M. C. Marchetto, and F. H. Gage. 2010. Review Mechanisms Underlying Inflammation in Neurodegeneration. *Cell*, 140(6), 918–934. <https://doi.org/10.1016/j.cell.2010.02.016>
- Gupta A, Kumar R, Ganguly R, Singh AK, Rana HK, Pandey AK. 2021. Antioxidant, anti-inflammatory and hepatoprotective activities of *Terminalia bellirica* and its bioactive component ellagic acid against diclofenac induced oxidative stress and hepatotoxicity. *Toxicology Reports*, 8, 44-52. doi:<https://doi.org/10.1016/j.toxrep.2020.12.010>
- Haidara M, Dénou A, Haddad M, Camara A, Traoré K, Aubouy A, *et al.* 2020. Evaluation of Anti-inflammatory, Anti-pyretic, Analgesic, and Hepatoprotective Properties of *Terminalia macroptera*. *Planta Medica International Open*, 07(02), e58-e67. doi:10.1055/a-1142-7072
- Harbourne, B. 1998. Free resolutions of fat point ideals on P2. *Journal of Pure and Applied Algebra*, 125(1-3), 213-234.
- Jami MSI, Sultana Z, Ali ME, Begum MM, Haque MM. 2014. Evaluation of Analgesic and Anti-Inflammatory Activities on Ethanolic Extract of <i>Terminalia chebula</i> Fruits in Experimental Animal Models. *American Journal of Plant Sciences*, Vol.05No.01, 7. doi:10.4236/ajps.2014.51010
- Kuete, V., T. K. Tabopda, B. Ngamei, F. Nana, T. E. Tshikalange, and B. T. Ngadjui. 2010. Antimycobacterial, antibacterial and antifungal activities of *Terminalia superba* (Combretaceae). *South African Journal of Botany*, 76(1), 125–131. <https://doi.org/10.1016/j.sajb.2009.09.009>
- Li, Z., L. Zhang, and Z. Zhao. 2021. Malylngamide F Possesses Anti-Inflammatory and Antinociceptive Activity in Rat Models of Inflammation. *Pain Research and Management*, v2021. pp 1-9. <https://doi.org/10.1155/2021/4919391>
- Lorke, D. 1983. A new approach to practical acute toxicity testing. *Archives of toxicology*, 54, 275–287.
- Lucas, S. M., N. J. Rothwell, and R. M. Gibson. 2006. The role of inflammation in CNS injury and disease. *British Journal of Pharmacology*, 147(SUPPL. 1), 232–240. <https://doi.org/10.1038/sj.bjp.0706400>
- Ma, K., Z. Zhu, C. Yu, H. Zhang, J. Liu, and L. Qin. 2011. Analgesic, anti-inflammatory, and antipyretic activities of the ethanol extract from *Desmodium caudatum*. *Pharm Biol*.

2011. 49 (4):403-7. <https://doi.org/10.3109/13880209.2010.520322>
- Mbiantcha, M., J. Almas, S. U. Shabana, D. Nida, and F. Aisha. 2017. Anti-arthritic property of crude extracts of *Piptadeniastrum africanum* (Mimosaceae) in complete Freund's adjuvant-induced arthritis in rats. *BMC Complementary and Alternative Medicine*, 17(1), 1–16. <https://doi.org/10.1186/s12906-017-1623-5>
- Moni, J. N. R., M. Adnan, A. M. Tareq, M. I. Kabir, A. S. M. A. Reza, M. S. Nasrin, K. H. Chowdhury, S. A. J. Sayem, M. A. Rahman, A. H. M. K. Alam, S. B. Alam, M. A. Sakib, K. K. Oh, D. H. Cho, and R. Capasso. 2021. Therapeutic potentials of *syzygium fruticosum* fruit (Seed) reflect into an array of pharmacological assays and prospective receptors-mediated pathways. *Life*, 11(2), 1–21. <https://doi.org/10.3390/life11020155>
- Moore A, S. Derry, C. Eccleston, E. Kalso. 2013. Expect analgesic failure; pursue analgesic success: *British Medical Journal*, 346. f2690. pp1-6. doi:10.1136/bmj.f2690
- Muganga R, Bero J, Quetin-Leclercq J, Angenot L, Tits M, Mouithys-Mickalad A, et al. 2020. In vitro Antileishmanial, Antitrypanosomal, and Anti-inflammatory-like Activity of *Terminalia mollis* Root Bark. *Planta Medica*, 87(09), 724–731. doi:10.1055/a-1260-6382
- Muruganathan, G., G. Sudheer Kumar, P. Sathya Chethan, and S. Mohan. 2013. Anti-Arthritic and anti-inflammatory constituents from medicinal plants. *Journal of Applied Pharmaceutical Science*, 3(4), 161–164. <https://doi.org/10.7324/JAPS.2013.3429>
- Mussarat, S., R. Ali, S., Mothana, R. A., Ullah, R., and M. Adnan. 2021. Medicinal Animals and Plants as Alternative and Complementary Medicine in Southern Regions of Khyber Pakhtunkhwa, Pakistan. *Frontiers in Pharmacology*, 12, 649046. pp1-17. <https://doi.org/10.3389/fphar.2021.649046>
- Navegantes, K. C., R. de Souza Gomes, P. A. T. Pereira, P. G. Czaikoski, C. H. M. Azevedo, and M. C. Monteiro. 2017. Immune modulation of some autoimmune diseases: the critical role of macrophages and neutrophils in the innate and adaptive immunity. *Journal of translational medicine*, 15(1), 1-21.
- Nesa, M. L., S. M. S. Karim, K. Api, M. R. Sarker, and M. Islam. 2018. Screening of *Baccaurea ramiflora* (Lour .) extracts for cytotoxic , analgesic , anti- inflammatory , neuropharmacological and antidiarrheal activities. *BMC complementary and alternative medicine* 18(1):35 1–9. <https://doi.org/10.1186/s12906-018-2100-5>
- Nworu C. S. and P. A. Akah. 2015. Anti-inflammatory medicinal plants and the molecular mechanisms underlying their activities. *Afr J Tradit Complement Altern Med* . (2015) 12 (Suppl .): 52-61
- Okoli, C. O., P. A. Akah, and U. Ezugworie. 2006. Anti-inflammatory activity of extracts of root bark of *Securidaca longipedunculata* Fres (Polygalaceae). *African Journal of Traditional, Complementary and Alternative Medicines*, 3(1), 54–63. <https://doi.org/10.4314/ajtcam.v3i1.31139>
- Osifo, M., S. A. Ihim, N. Ani, C. S. Nworu, and P. Akah. 2022. Wound healing and anti-inflammatory activities of *Ceiba pentandra* (L.) Gaertn. *Pharmacological Research - Modern Chinese Medicine*, 3, (p100077) pp 1-11. <https://doi.org/10.1016/j.prmcm.2022.100077>
- Patil, K. R., U. B. Mahajan, B. S. Unger, S. N. Goyal, S. Belemkar, S. J. Surana, S. Ojha, and C. R. Patil. 2019. Animal models of inflammation for screening of anti-inflammatory drugs: Implications for the discovery and development of phytopharmaceuticals. *International Journal of Molecular Sciences*, 20(18). Pp4367. <https://doi.org/10.3390/ijms20184367>
- Pokuri VK, Kumar CU, Pingali U. (2016). A randomized, double-blind, placebo-controlled, cross-over study to evaluate analgesic activity of *Terminalia chebula* in healthy human volunteers using a mechanical pain model. *Journal of Anaesthesiology, Clinical Pharmacology*, 32(3), 329–332. doi:10.4103/0970-9185.173365
- Rathi, A., C. V. Rao, B. Ravishankar, S. De, and S. Mehrotra. 2004. Anti-inflammatory and anti-nociceptive activity of the water decoction *Desmodium gangeticum*. *Journal of ethnopharmacology*, 95(2-3), 259-263.
- Rauf, A., N. Jehan, and M. S. Mubarak. 2017. Analgesic Potential of Extracts and Derived Natural Products from Medicinal Plants. World ' s largest Science , Technology & Medicine Open Access book publisher. March 2023. <https://doi.org/10.5772/intechopen.68631>
- Reuter, S., S. C. Gupta, M. M. Chaturvedi, and B. B. Aggarwal. 2010. Oxidative stress, inflammation, and cancer: How are they linked? *Free Radical Biology and Medicine*, 49(11), 1603–1616. <https://doi.org/10.1016/j.freeradbiomed.2010.09.006>
- Singh, B., and R. A. Sharma. 2013. Anti-inflammatory and antimicrobial properties of pyrroloquinazoline alkaloids from *Adhatoda vasica* Nees. *Phytomedicine*, 20(5), 441–445. <https://doi.org/10.1016/j.phymed.2012.12.015>
- Singh P, K. Kongara, D. Harding, N. Ward, V. S. R. Dukkupati, and C. Johnson. 2018. Comparison of electroencephalographic changes in response to acute electrical and thermal stimuli with the tail flick and hot plate test in rats administered with opiorphin. *BMC Neurology*, 18(1), 43. 1 -10 doi:10.1186/s12883-018-1047-y
- Stollenwerk A, M. Sohns, F. Heisig, C. Elling, D. Von Zabern. 2018. Review of Post-Marketing Safety Data on Tapentadol, a Centrally Acting Analgesic. *Advances in Therapy*, 35(1), 12-30. doi:10.1007/s12325-017-0654-0
- Su, J. Y., Q. C. Li, and L. Zhu. 2011. Evaluation of the in vivo anti-inflammatory activity of a flavone glycoside from *Cancrinia discoidea* (Ledeb.) Poljak. *EXCLI journal*. 10, pp110.
- Tsai, D. S., M. H. Huang, J. C. Tsai, Y. S. Chang, Y. J. Chiu, Y. C. Lin, and W. H. Peng. 2015. Analgesic and anti-inflammatory activities of *Rosa taiwanensis* nakai in mice. *Journal of medicinal food*, 18(5), 592-600.
- Turner, N. C., F. M. Li, Y. C. Xiong, and K. H. Siddique. 2011. Agricultural ecosystem management in dry areas: challenges and solutions. *Plant and soil*, 347, pp1-6.