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Evaluation of Anti-inflammatory and Analgesic Activities of *Tithonia diversifolia* in Experimental Animal Models

A. O. Sijuade[1,2*], J. O. Fadare[1] and O. A. Oseni[3]

[1]Department of Pharmacology, College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria.
[2]Department of Biochemistry, Faculty of Natural and Agricultural Sciences, University of Pretoria, Hatfield, Pretoria 0083, South Africa.
[3]Department of Medical Biochemistry, College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AOS designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author JOF managed the literature searches, analysed the data. Author OAO managed the experimental process and editing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The common non-steroidal anti-inflammatory drugs (NSAIDs) and opioids have adverse effects. This has motivated for the search of new drugs with decreased or no side effects. In the present study, we investigated analgesic and anti-inflammatory activities of the methanol extract of *Tithonia diversifolia in vivo*.
Methods: Analgesic activity of the methanol extract of *T. diversifolia* was carried out using an analgesy meter to measure the tolerance of induced pressure. The method of carrageenan-induced inflammation in rat was used for the anti-inflammatory effect.
Results: This study shows statistically significant improvements in pain resistance and paw oedema suppression were observed in animals treated with 150 and 300 mg/kg body weight (b.w.)

**Corresponding author: E-mail: sijuade@hotmail.com;*

methanol extract of *Tithonia diversifolia,* when compared to control groups treated with normal saline and acetyl salicylic acid. The maximum analgesic effect was achieved at 300 mg/kg after 30 min with effective ratio of 5.92 when compared with the standard drug acetyl salicylic acid, and twice the activity of 150 mg/kg at the same time.

Conclusion: The results suggest that the plant extract has analgesic and anti-inflammatory activities, supporting its uses in traditional medicine.

Keywords: Tithonia diversifolia; plant extract; analgesics; anti-inflammatory; mice.

1. INTRODUCTION

Tithonia diversifolia (Hemsley) A. Gray (Asteraceae: Heliantheae), which is also called Mexican sunflower, is a shrub which originated from Mexico and Central America. This was later introduced into Africa, Australia, Asia and South America [1]. It is a bushy perennial weed commonly found on the fields, wastelands and road sides of Nigeria. The plant has many applications apart from its medicinal values, it has been used for ornamental purposes, as manure and for treatment of diabetes mellitus. The plant extract has been used in medicine for its diverse healing power. In Nigeria, this plant has been linked to treatment of pain [2]. Likewise, in Kenya, the plant is commonly used for the treatment of stomach pains, sore throats and liver ailments [3].

Pharmacologically, the plant has been reported for anti-inflammatory, anti-diarrhea, anti-amoebic, spasmolytic and antimalarial activities [4,5,6]. The presence of phytochemicals such as flavonoids, steroids, lignans, polyphenols, coumarins, terpenes and alkaloids in medicinal plants are scientifically proven to relieve inflammation, pain and fever. Several other reports have been made on medicinal plants with analgesic, anti-inflammatory and antidiabetic properties [7-10]. Baruah et al. [11] reported the insecticidal property of the *T. diversifolia* containing tagitinin A–C and F with tagitinin A. and isolation of some other chemicals such as diversifol, tithonine, and sulphurein. The tagitinin C, a sesquiterpene lactone from the plant has been reported to exhibit antiplasmodial activity and possessed cytotoxic properties *in vitro* [1].

Pain is one of the most common reasons patients visit hospitals for physician consultation in most developed countries [12,13]. It is a major symptom for diagnosing illness in many medical conditions, and can interfere with person's quality of life and general function of the body [14]. Despite the extensive research work on drug discovery, there is still a gap in the development of a safe, effective and economical therapy for managing chronic inflammation and pain. In particular, the adverse cardiovascular and gastrointestinal side effects associated with long term use of selective or non-selective NSAIDs re-enforces the need to develop new drug from medicinal plants with anti-inflammatory and analgesic activities without the side effects that accompany NSAIDs [15,16]. Pain can be inflammatory or neuropathic. Inflammatory pain emanates from chemical and physical stimuli through damaged tissue, and central pain is caused by direct lesions or sensory nerve disease. Many factors such as TNF-α, PGE2, 5-HT, and NF-κB are involved in inflammatory or central pain [17,18]. This study evaluated the anti-inflammatory and anti-nociceptive properties of *T. diversifolia in vivo*.

2. MATERIALS AND METHODS

2.1 Plant Collection

Tithonia diversifolia whole plant (except the root) was collected from surrounding of Ekiti State University, Ado-Ekiti, Nigeria. The identification and authentication of the plant was done at the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria. This was later confirmed at the Forestry Research Institute, Ibadan, Nigeria.

2.2 Preparation of the Plant Extract

The preparation of the plant extract was done at the Department of Pharmacology, Ekiti State University, Ado-Ekiti, Nigeria. Freshly collected *T. diversifolia* (TD) plant was separated from adhering materials such as weed, and rinsed with distilled water. Plants were shade-dried for two weeks before they were ground into a coarse powder with a kitchen blender. The powder was kept in a dry, cool and dark place in an airtight container. Approximately, 400 g of powdered *T. diversifolia* was mixed with 900 mL of 95% methanol in a clean, flat-bottomed glass container. The container was sealed and kept for

a week with occasional shaking. Coarse plant material was separated from the mixture by pouring through a clean filter. This extract was filtered, and the filtrate was concentrated in vacuo to dryness at 40℃ using a rotary evaporator. The extract yield was 13.8%. This was stored at 4℃ until used. The extracts were dissolved in 0.9% NaCl solution to the desired concentration just before use.

2.3 Experimental Animals

Swiss albino mice of mixed sexes weighing between 25-30 g and Albino Rat (150-200 g) bred from the experimental Animal House of College of Medicine, Ekiti State University, Nigeria were used for the experiment. The animals were kept in cages within the animal house and allowed free access to water and standard livestock pellets. They were examined and found to be free of wounds, swellings and infections before the commencement of the experiment. All experimental procedures were conducted in accordance with accepted standard guidelines of National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication no. 85–23, revised 1985). All experiments were conducted in the Research Laboratory of Department of Pharmacology, College of Medicine, Ekiti State University, Nigeria.

2.4 Chemicals and Reagents

Chemicals used include carrageenan from Sigma-Aldrich Chemie Gmbh (Steinheim, Germany). All the chemicals and drugs used were of analytical grade. Methanol (Merck, Germany), Normal saline (0.9% Sodium chloride) and Acetyl salicylic acid (Aspirin) or ASA.

2.5 Experimental Design

2.5.1 Determination of analgesic activity

2.5.1.1 Carregeenan induced paw oedema

An injection 0.1 ml of 1% (w/v) carrageenan was applied into the sub-plantar tissue of rat left hind paw to induce pedal inflammation according to the method described elsewhere [19-21]. The control group (I) received 10 ml/kg normal saline and the reference drug group (II) received 10 mg/kg indomethacin (Strides, Belgium) orally. The test groups (III and IV) of rats were treated orally with 150 or 300 mg/kg of the extract, respectively, 1 h before carrageenan injection. The level of inflammation induced by the carrageenan was determined by measuring the diameter of the paw at 0 min, 30, 60, 90 and 120 min after the administration of carrageenan using a micrometer screw gauge. The anti-inflammatory effect of the extract and reference drug was calculated from the formula

$$\% \text{ Inhibition} = (X_0 - X_t) / X_0 * 100$$

where X_0 was the average inflammation, i.e., mean paw size, of the control group, and X_t was the average inflammation, i.e., mean paw size, of the drug-treated groups (indomethacin and plant extract).

2.5.1.2 Hot plate test

Mice were randomly divided into the following 5 groups of 5 mice each: (I) a vehicle group (Normal Saline, NS), (II) aspirin group (15 mg/kg ASA), and (III and IV) two *Tithonia diversifolia* extract groups (150 or 300 mg/kg). Anti-nociceptive drug activity was assessed using the hot plate test as previously reported [22]. The temperature of the metal surface was maintained at 55.0±0.5℃. The latency between the placement and shaking or the licking of the hind paws or the jumping response of the animals was recorded as the latent response. The mice were screened in advance, and a pre-test latency of 5–30 s qualified them for the experiment. The mice were treated with NS (10 ml/kg, p.o.) or TD extract (150 or 300 mg/kg, p.o.) 30 min before the test. Aspirin (15 mg/kg, p.o.) was used as a positive control drug and was administered 30 min before the experiment. A 60-s cut-off time was used to minimize tissue damage in the mouse. Latencies were measured prior to treatment and at 30, 60, 90, and 120 min after drug administration.

2.5.1.3 Pressure test using analgesy meter

Mice were randomly separated into 4 groups of 5 as follows:

Group I, the control group, received 0.2 ml of normal saline. ***Group II***, the positive control group, was treated with standard drug aspirin at 15 mg/kg body weight. ***Group III***, test group, administered with 150 mg/kg body weight (b.w) extract. ***Group IV,*** test group, administered with 300 mg/kg b.w. extract respectively. Treatments (control vehicle, extracts and standard drug) were administered orally 30 min prior to pressure test.

In these experiments, different doses of the extract and standard drug were tested in mice using an Ugo Basile Analgesy Meter (N° 7200). A constant force was applied to the left hind paw of experimental animals by the Analgesy Meter plunger. Mice were restrained in the upright position while their left hind paws were placed between the plinth and the plunger. Pain was determined by the physical struggles of the animal to set itself free. The weight causing pain before treatment was used at time intervals: 30 min, 60, 90,120 and 180 min after treatment of animals with the various doses. The time at which the animal starts physical struggling to free itself was recorded.

2.6 Statistical Analysis

GraphPad Prism Version 5 software was used for statistical analysis. Values are expressed as mean±S.E.M. Student's *t*-test was carried out to compare the results of control and test drug groups. Data were considered to be significant when P<0.05.

3. RESULTS

3.1 Carrageenan-induced Paw Oedema in Rats

The results of the anti-inflammatory and analgesic activities of the methanol extract of *T. diversifolia* are presented in Tables 1 and 2. Using the carrageenan method, the size of the oedema reduced from 4.2±0.05 to 0.62±0.01 mm in all the test groups. There was a significant difference between the effect of the doses of the extract and control (NS) throughout the study frame time (p<0.001). The effect of the extract at 300 mg/kg and that of the reference drug were pronounced at 90 min after carrageenan injection, while that of 150 mg/kg was highest at 120 min after carrageenan injection. The effect of the extract (150 mg/kg) was not as pronouced as that of the Indomethacin between 60-120 min (p<0.05) (Table 1).

3.2 Analgesy Meter Test

The analgesic activity of the methanol extract of *Tithonia diversifolia*, normal saline, acetyl salicylic acid evaluated using analgesy meter method are given in Table 2. The extract exhibited good analgesic effect up to 120 min at a dose of 300 mg/kg and 150 mg/kg as compared to control groups. The maximum analgesic effect was achieved at 300 mg/kg after 30 min with effective ratio of 5.92 when compared with the standard drug acetyl salicylic acid, and twice the activity of 150 mg/kg at the same time (Fig. 1). The methanol extract has a higher analgesic effect than ASA, the reference drug used in this study (Table 2).

Table 1. Anti-inflammatory activity of the methanol extract of *T. diversifolia* (150 mg/kg and 300 mg/kg) and indomethacin on carrageenan-induced oedema in the left hind limb of rats

Test groups	Paw oedema (mm)				
	0 min	30 min	60 min	90 min	120 min
Normal saline (NS)	2.47±0.30	2.90±0.10	3.43±0.30	3.93±0.23	4.20±0.05
Indomethacin 10 mg/kg	2.67±0.33	1.83±0.03	0.87±0.09	0.60±0.06	0.46±0.03
T. diversifolia 150 mg/kg extract	2.47±0.27	1.80±0.06*	1.20±0.07*$	0.80±0.04*$	0.63±0.03*$
T. diversifolia 300 mg/kg extract	2.53±0.23	1.87±0.09*¶	0.97±0.03*¶	0.61±0.01*¶	0.47±0.05*$

**p<0.001 extract and control (NS), ¶ p>0.05, $P<0.05, extract and Indomethacin.*

Table 2. Analgesic activity of *T. diversifolia* tested by analgesy-meter method in mice

	Reaction time (Seconds)				
Test groups	0 min	30 min	60 min	90 min	120 min
Normal Saline	6.30±0.17	4.17±0.88	5.83±0.67	5.50±0. 76	5.50±0.29
ASA 15 mg/kg	6.67±0.44	7.23±0.15	8.50±0.76	10.33±2.40	11.33±0.66
T. diversifolia 150 mg/kg extract	6.57±0.70	10.70±1.67	12.83±1.30	14.33±2.40	15.00±0. 57*
T. diversifolia 300 mg/kg extract	7.53±0.26	15.50±2.30*	19.50±2.30*	17.50±3.01*	19.00 ±1.26*

*n=5, *: P<0.01 as compared to ASA (significant).*

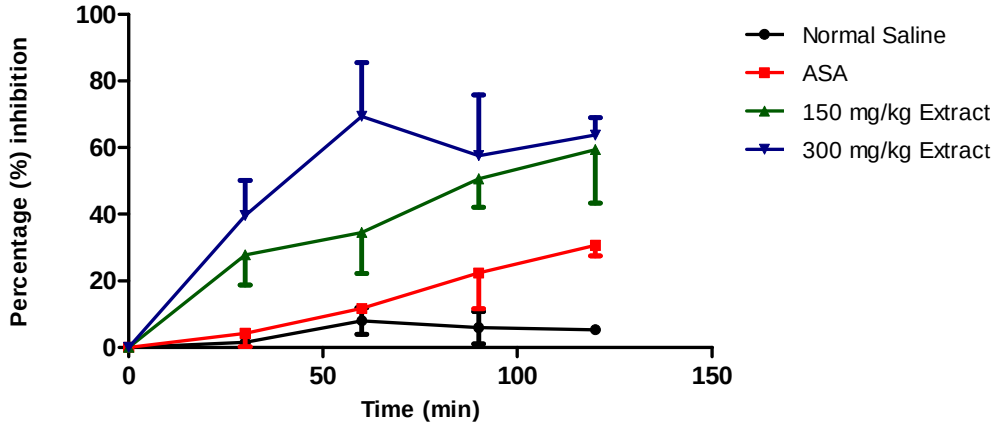

Fig. 1. Effect of methanol extract of *T. diversifolia* and Acetyl salicylic acid (ASA) on pressure of mice paw test. After drug administration, the reaction time of mice to analgesy-meter was measured prior to treatment and after that. Each point represents percentage inhibition (mean±SEM) of the reaction time for n=5 mice

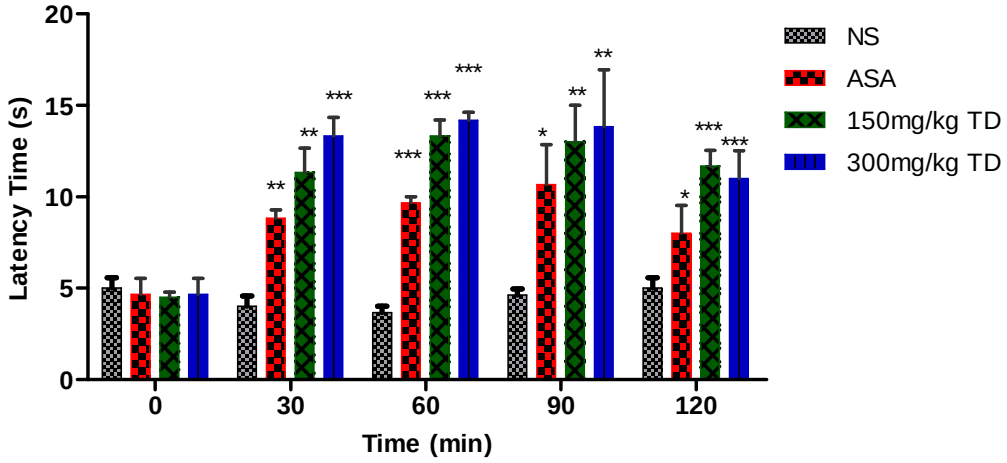

Fig. 2. Effects of methanol extract of *T. diversifolia* on hot plate analgesic test in mice. Vehicle control mice were administered with normal saline and ASA (15 mg/kg) was used as the positive control. Results are expressed as the mean±SEM (n=5) of the reaction time in seconds

p<0.05,p<0.01,***p<0.001 were considered significant when compared at the same time with the vehicle control (Normal saline) after administration*

3.3 Hot Plate Method

Results of analgesic activity of acetyl salicylic acid and treatment doses of *Tithonia diversifolia* measured by hot plate method are given in Fig. 2. The methanol extract of *T. diversifolia* exhibited good analgesic effect at two doses used (150 and 300 mg/kg) as compared to control and standard drug ($p<0.05$). Maximum analgesic effect was noted at a dose of 300 mg/kg at 60 min after administration of the extract as compared to control and standard drug.

4. DISCUSSION

Traditional medicines derived from medicinal plants are used by over 50% of the world's population making the consumption of traditional medicine to be very common nowadays People

are taking it as either in form of food supplement or raw for the treatment of one ailment or the other. In another view, it is also consumed as liquor drinks in some part of West Africa, especially, Nigeria. Approximately 21,000 plants has been listed by the World Health Organization (WHO) for medicinal purposes around the world.

This study was able to evaluate the response of experimental animal when exposed to heat and mechanical stimuli and carrageenan administration as a measure of anti-nociceptive and anti-inflammatory effects of the *T. diversifolia* plant extract. The methanolic extract of the plant has shown analgesic and anti-inflammatory properties. A similar study was also conducted by Owoyele et al. [2] but did not study pain using mechanical pressure. However this study, used a standard mechanical pressure-induced model, analgesy meter. The mechanical pressure form of pain was selected to mimic human accidental pain or mechanically induced pain."

Carrageenan, a phlogistic agent that is widely used to induce inflammation in experimental animal for the screening of plants that possess anti-inflammatory activity [23,24]. It has a biphasic effect during inflammation induction. Carrageenan was used to induce inflammation in this study and the induced oedema involves the synthesis or release of mediators at the injured site. Such mediators include prostaglandins, especially the E series, histamine, bradykinins, leukotrienes and serotonin, all of which also cause pain and fever [25]. Thus, the extract was able to reduce oedema significantly as evidence by its anti-inflammatory activity. Studies have reported phytochemicals such as alkaloids and flavonoids are responsible for the anti-inflammatory and anti-nociceptive activity of prostaglandin synthetase inhibition, prostaglandins which are observed in the late phase of acute inflammation and pain perception [26]. In addition, it has been reported that carrageenan-induced paw oedema test is effectively controlled with the arachidonate cyclooxygenase (COX) inhibitors due to its COX-dependent mechanism, thus, from the observed results, it is suggested that *T. diversifolia* may possess arachidonate COX inhibitory property [27].

In the hot plate test, ASA and the two doses of extract displayed marked anti-nociceptive effects. The analgesic effect of ASA was similar ($p>0.05$) with other two doses of extract at 90 min post-treatment. However, at 60 min post-treatment, the high and lower doses of the extract displayed a stronger effect on the nociceptive threshold than ASA, although the effects of the extract did not show a dose-dependent response. Hence, anti-inflammatory and analgesic activities of the methanolic leaf of the plant extract may be attributable to the existence of alkaloids and flavonoids either in single form or in combination. And previous studies have shown the presence of these phytochemicals in *T. diversifolia* extract [28]. These analgesic models suggested that the analgesic effect of *T. diversifolia* extract may be mediated by inhibiting the synthesis and release of prostaglandins and other pro-inflammatory cytokines such as IL-1, IL-6, IL-8, and TNF-α [8,29].

The major challenge is that the plant has been shown to possess both haematological and acute toxic effects on the kidney and liver though, these effects are time and dose dependent [30]. The group also showed that repeated administration of the extract at high doses (≥400 mg/kg/day) could cause irreversible damage to the kidney and liver organs and the LD_{50} was found to be greater than 1600 mg/kg/day in the toxicity test. The doses used in this study are lower, not lethal and there was no sign of toxicity during the study. However, care must be taken when using the plant as home concussion.

5. CONCLUSION

T. diversifolia leaf extract exhibited significant anti-inflammatory and analgesic activities. Thus, the present study validates the traditional use of *T. diversifolia* in treatment of pain and inflammatory related disorder. However, further studies are warranted to elucidate the exact mechanism of action and confirm the chemical constituents responsible for the anti-inflammatory and analgesic effects of *Tithonia diversifolia.*

CONSENT

It is not applicable.

ETHICAL APPOVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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