

---

## **ANTI-INFLAMMATORY AND ANALGESIC POTENTIAL OF AQUEOUS LEAF EXTRACT OF *TITHONIA DIVERSIFOLIA* IN RODENTS**

**\*<sup>1</sup>J.O. OLUKUNLE, <sup>2</sup>E.A.O SOGEBI AND <sup>3</sup>J.AOYEWUSI**

1. Department of Veterinary Physiology and Pharmacology, Federal University of Agriculture, Abeokuta, Nigeria.

2. Department of Veterinary Medicine and Surgery, Federal University of Agriculture, Abeokuta, Nigeria.

3. Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Nigeria.

**\*Corresponding author:** drfaks@yahoo.com **Tel:** +234810184 6078

---

### **ABSTRACT**

This study was carried out to assess the anti-inflammatory and analgesic potentials of aqueous extract of *Tithonia diversifolia* leaves in laboratory animals. Anti-inflammatory and analgesic effects of aqueous extract of *T. diversifolia* leaves in laboratory animals were determined using carrageenan induced acute oedema model in rats, thermal and acetic-acid induced writhing tests were assessed in mice. Potentials of aqueous extracts of *Tithonia diversifolia* leaves at 400 mg/kg was assessed in rats and mice, which were compared with controls; a negative control given 10ml/kg distilled water, positive control groups administered with Indomethacin, aspirin, paracetamol and morphine. The mean percentage inhibition of increase in paw volume was highest in rats to which indomethacin was administered (85.65%), followed by rats administered aspirin (68.40%) which was comparable with that of rats dosed with 400mg/kg of *Tithonia diversifolia* leave extract (63.79 %). Also, extract increased significantly ( $P < 0.05$ ) the reaction time in the hot plate test ( $25.18 \pm 0.7^*$ ) /s and the percentage inhibition of writhing of (43.92%) was significant at ( $P < 0.05$ ) in groups dosed with the extract and highly comparable with the performance of paracetamol (49.64%) a standard analgesic agent. The study established the anti-inflammatory and analgesic potential of *Tithonia diversifolia* aqueous leaf extract.

**Keywords:** *Tithonia diversifolia*, extract, anti-inflammatory, analgesic.

### **INTRODUCTION**

Inflammation is a protective response elicited to tissue injury or destruction, it serves to destroy, dilute, or wall off both the injurious agent and the injured tissue. It is a way by which the body removes the injurious stimuli as well as initiates the healing process (Denko, 1992). The injury can be caused by physical, chemical, and biologic agents, including mechanical trauma. Exposure to excessive amount of sunlight, x-rays, radio-

active materials, corrosive chemicals, extremes of heat and cold, or by infectious agents such as bacteria, viruses, and other pathogenic microorganisms can also lead to inflammation.

This inflammatory response leads to the activation of the body defense cells, the release of complements, cytokines, prostaglandins and other inflammatory mediators (Cotran *et al.*, 2001). The classical signs of inflammation

are heat, redness, swelling (oedema), pain and loss of function.

Pain is a regular occurrence in most cases of acute and chronic inflammatory diseases and any good therapeutic protocol for inflammatory diseases will take into consideration the anti-inflammatory and analgesic capabilities.

Major limitations to the use of standard anti-inflammatory drugs are failure of therapy, undesirable side effects like gastric ulcerations and high cost of the therapy (Rainsford and Whitehouse, 1980). This indicates a great need for the development of alternative strategies for prevention and treatment of inflammatory diseases. Natural products play an important role in drug development programs in the pharmaceutical industry.

Plants are the basic source of knowledge of modern medicine and especially phytomedicines are adequate nutritional supplements, which would be of direct benefits to patients.

Many plants have long been recognized as important sources of therapeutically effective medicines (Cragg *et al.*, 2003). Herbs such as *Tridax procumbens*, *Jatropha curcas* and *Morinda morindoides* have been shown to possess anti-inflammatory activities (Diwan *et al.*, 1989, Owoyele *et al.*, 2003, Olukunle *et al.*, 2011; Olukunle *et al.*, 2012), while many others have also exhibited their analgesic potentials (Vohora *et al.*, 1997). Medicinal plants of this type can be useful as sole therapeutic agent or as an adjunct to standard drugs in managing inflammatory conditions.

*Tithonia diversifolia* is renowned in most of the West Africa nations for its potent anti-

malarial and repellent activities (Oyewole *et al.*, 2008) and is also a plant that has been commonly used for diverse medicinal purpose in many countries of the world. *Tithonia diversifolia* has been reported to have curative effect against scabies in rabbits (Hang *et al.*, 2012). Goffin *et al.*, (2002) reported its antiplasmodial activity in a study which was confirmed by Madureira *et al.*, (2002) who reported the activity of *T. diversifolia* against chloroquine resistant *plasmodium falciparum*. Bork *et al.*, (1996) also observed that *T. diversifolia* possesses anti-inflammatory and antibacterial activities. Jayawardena *et al.*, (2000) reported the anthelmintic potential of *T. diversifolia* leaf extract and this was corroborated by the work of Hamowia and Saffaf, (1994), who reported its anti-tumor activity. The work of Miura *et al.*, (2002) showed the antidiabetic potential of *T. diversifolia* and that it significantly improved the blood glucose level of diabetic KK-Ay mice by reducing insulin resistance.

Although the use of many of these herbal plants like *T. diversifolia* in traditional practice have a rationale background, it is essential to investigate the rationality of their use in modern scientific terms. This study is aimed at investigating the scientific rationale behind the anti-inflammatory and analgesic usage of *Tithonia diversifolia* as claimed by folklore.

## MATERIALS AND METHODS

**Animals:** Twenty four mature Wistar albino rats (*Rattus norvegicus*) of both sexes weighing 150-200g and thirty six Swiss mice (*Mus musculus*) of both sexes weighing 25-30g were used for this study.

The animals were obtained from the Laboratory Animal Unit, Department of Physiology, University of Ibadan, Ibadan.

The animals were kept at the Experimental Animal House, College of Veterinary Medicine, University of Agriculture, Abeokuta.

They were provided with pelletized feed (Vital feeds Limited, Ibadan, Nigeria) and water *ad-libitum*. The animals were kept for two weeks prior to the commencement of the study for acclimatization to laboratory conditions.

The housing provided had the following conditions; controlled lighting of 12:12 hours of light: dark, temperature of 25°C and relative humidity of approximately 50%.

**Plant material:** Fresh leaves of *T. diversifolia* (Asteraceae) were collected from Odeda Local Government Area of Ogun State, Nigeria and were identified at the Botany Department of the Federal University of Agriculture, Abeokuta and also at the Forestry Research Institute of Nigeria (FRIN), Ibadan by Mr. Ekundayo.

**Plant extract:** The plant extract was prepared according to the method by Olukunle *et al.*, (2012). Fresh leaves were collected and air-dried in the laboratory at the room temperature (27°C) for two weeks. The leaves were pulverized, sieved and 1000g was soaked in 2liters of distilled water for 24hrs. The filtrate was freeze dried and 2gm of it was dissolved in 20ml of distilled water to give a concentration of 100mg ml<sup>-1</sup>.

**Drugs:** Commercial preparations of the following drugs were used for this study- Aspirin at 150mg/kg (100mg tablet of Acetylsalicylic acid, Jubel Nigeria Limited), Indomethacin at 10mg/kg, (Greenfield Pharmaceuticals Nigeria Limited) and 50mg/kg of Paracetamol (500mg tablet), May and Baker Nigeria Plc, dissolved in water were

administered orally to the animals while 15mg/ml of Morphine Sulphate (Antigen Limited, Ireland) and 0.1ml (1% suspension of carrageenan in normal saline) Carrageenan (Sigma-Aldrich, Belgium) were administered intraperitoneally to the animals.

All experimental protocols carried out on the animals were in accordance with the internationally accepted principles for the laboratory animal usage and were approved by Ethics Committee on the Laboratory Animal Use of the College of Veterinary Medicine of University of Agriculture, Abeokuta.

#### **Anti-inflammatory test**

##### **Carrageenan-induced rat paw oedema:**

Acute inflammation was induced using the carrageenan induced oedema model (Winter *et al.*, 1962). The rats were starved for 12 hours after which they were divided into four groups (Groups A-D) of six animals each.

Group A rats which were the negative control (untreated) were given 10ml/kg distilled water administered one hour before 0.1ml of 1% freshly prepared suspension of carrageenan was injected into the plantar surface of the right hind paw of each rat. Group B rats were given aqueous leaf extract of *T. diversifolia* orally at the dose of 400mg/kg one hour before carrageenan injection. Rats in Group C were given aspirin, a standard anti-inflammatory drug at the dose of 150mg/kg orally one hour before carrageenan injection while Group D rats were administered indomethacin, another standard anti-inflammatory drug at a dose of 10mg/kg orally one hour before carrageenan injection, both serving as positive control.

The linear circumference of the paw was measured at zero hour on injecting the carrageenan injection and three hours after carra-

geenan injection using a loop of thread tied round the paw such that it was neither too loose nor too tight. The length of the thread round the paw was then measured on a rul-

er and the readings were repeated three times and the average taken to avoid errors. The percentage inhibition was calculated according to the formula:

$$\text{Eq. (A.1), Percentage Inhibition} = \frac{[C1-C0] \text{ control} - [C1-C0] \text{ test}}{[C1-C0] \text{ control}} \times 100$$

where Co= Mean paw size at the 0 hour of carrageenan administration.  
C1= Mean paw size at 3 hours after the carrageenan administration <sup>7</sup>.

### Anti-nociceptive tests

**Hot plate test:** This was done as described by Hikino *et al.*, (1985). The mice were divided into three groups of six mice each. The mice in group 1 were administered with aqueous leaf extract of *T. diversifolia* (400mg/kg), mice in group 2 were dosed 2mg/kg morphine while those in the group 3 which was the control were given 10ml/kg of distilled water. The treatment was done one hour before the mice were placed on a hot plate (55 ± 1°C) and the reaction time (licking one of the hind paws and jumping off the plate) to thermal stimulus (pain) was observed within 1 minute.

### Acetic acid-induced abdominal writhing in mice:

This was done as described by Koster *et al.*, (1959). The mice were divided into three groups (Groups I- III) of six mice each. Distilled water at 10ml/kg orally was administered to the mice in the control group (Group I), 50mg/kg paracetamol orally to Group II animals and 400mg/kg of aqueous leaf extract of *T. diversifolia* was given orally to mice in Group III one hour before intraperitoneal injection of acetic acid (0.6%, v/v in distilled water at 10ml/kg). The number of writhes exhibited by each animal was counted for 10 minutes beginning 10 minutes after acetic acid injection and computed according to the following formula

Eq. (A.2)(Olukunle *et al.*, 2011),

$$\text{Percentage Inhibition} = \frac{\text{Mean of writhes}(\text{control}) - \text{Mean of writhes}(\text{test})}{\text{Mean number of writhes}(\text{control})} \times 100$$

**Statistical analysis:** Results were expressed as mean ± SEM. Analysis of the data was done using the one-way Analysis of Variance (ANOVA) followed by the Duncan multiple range test. P value < 0.05 was considered significant in all cases.

## RESULTS

**Anti-inflammatory test:** Aqueous leaf extract of *T. diversifolia* at a dose of 400mg/kg exhibited significant anti-inflammatory activity in carrageenan induced rat paw oede-

ma model.

The mean percentage inhibition of paw volume was highest in rats to which indomethacin was administered (85.65%), followed by the rats administered aspirin (68.40%) which was comparable with that of rats given *Tithonia diversifolia* (63.79%)(Table 1). The implication of this is that indomethacin caused the highest inhibition of paw oedema, followed by inhibition caused by aspirin and that caused by *T. diversifolia* extract.

**Table 1: Effect of aqueous extract of *T. diversifolia* on carrageenan induced paw oedema in Wistar rats**

Groups (n=6)	Dose mg/kg p/o	CBF (cm) 0hr	CAF (cm) 3hr	% inhibition in rat paw volume
Group A (CONTROL)	10	2.80 ± 0.47	3.38 ± 0.04	13.09 ± 3.71
Group B ( <i>T. diversifolia</i> )	400	2.82 ± 0.10	3.03 ± 0.10	63.79±12.00*
Group C (Indomethacin)	10	2.88 ± 0.03	2.96 ± 0.03	85.65±13.40*
Group D (Aspirin)	150	3.03 ± 0.02	3.23 ± 0.42	68.40±10.23*

\* P < 0.05, Values are Mean ± SEM

CBF – Circumference of the rat paw immediately after carrageenan injection (cm).

CAF - Circumference of the rat paw 3hours after carrageenan injection (cm).

#### **Anti-nociception tests**

**Hot plate test:** Aqueous leaf extract of *T. diversifolia* at a dose of 400mg/kg also exhibited significant anti-nociceptive activity in mice (Table 2).

The group dosed with morphine had the highest reaction time of (31.81± 1.50)/s,

followed by the extract which caused a significant (P<0.05) increase in reaction time of (25.18 ± 0.70)/s in the hot plate test when compared to the untreated control reaction time of (18.22 ± 0.27)/s indicating an increase in pain threshold level.

**Table 2: Effect of aqueous extract of *T. diversifolia* on hot plate-induced pain in mice**

Groups (n=6)	Dose mg/kg p/o	Reaction time (sec)
Group I (distilled water)	10	18.22 ± 0.27
Group II (Morphine)	50	31.81 ± 1.50*
Group III ( <i>T. diversifolia</i> )	400	25.18 ± 0.7*

\*P<0.05, Values are Mean ± SEM

**Acetic acid- induced abdominal writhing in mice**

The mean number of writhing movements was significantly lower ( $P < 0.05$ ) in mice dosed with 400mg/kg aqueous leaf extract of *T. diversifolia* ( $26.17 \pm 0.7$ ) culminating in a (43.92%) inhibition of writhing when

compared with the negative control ( $46.7 \pm 1.4$ ). Though this was higher than the writhing values in mice that was administered with the standard analgesic agent, paracetamol ( $23.5 \pm 1.3$ ) with percentage inhibition of (49.64%) (Table 3).

**Table 3: Effect of aqueous extract of *T. diversifolia* on acetic acid-induced writhing in mice**

Groups (n=6)	Dose mg/kg p/o	Number of writhes (per 10 min.)	% Inhibition of writhes
Group I (Control)	5ml/kg (distilled water)	$46.67 \pm 1.36$	0.00
Group II (Paracetamol)	50	$23.50 \pm 1.28$	49.64*
Group III ( <i>T. diversifolia</i> )	400	$26.17 \pm 0.7$	43.92*

Values are Mean  $\pm$  SEM

\*Statistically significant at  $P < 0.05$

## DISCUSSION

This study has evaluated for the anti-inflammatory and anti-nociceptive activities of aqueous leaf extract of *Tithonia diversifolia*. Our result suggests that aqueous leaf extract of *T. diversifolia* has anti-inflammatory effect comparable with those of the standard drugs, aspirin and indomethacin.

Carrageenan-induced inflammatory process is believed to be biphasic (Vinegar *et al.*, 1969). The initial phase seen at the 1<sup>st</sup> hour is attributed to the release of histamine and serotonin (Cruckhon and Meacock, 1971). The second accelerating phase of swelling is due to the release of prostaglandin, bradykinin and lysozyme. Katzung, (1998), reported that the second phase of oedema is

sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents.

The anti-inflammatory activity exerted by the leaf extracts of *Tithonia diversifolia* suggests that it could have acted by affecting the release of these inflammatory mediators. Chagas-Paula (2003), reported that chlorogenic acids from *Tithonia diversifolia* demonstrated better anti-inflammatory effects than standard agent indomethacin.

The presence of flavonoids in plants which has been shown to be part of the constituents of *Tithonia diversifolia* (Hang *et al.*, 2012; Uduak and Nodeley, 2013) is believed to be the factor responsible for the anti-inflammatory effects because flavonoids are known to exert strong anti-inflammatory

activity (Alejandra *et al.*, 2003).

An important effect of flavonoids is the scavenging of oxygen-derived free radicals (Van Acker *et al.*, 1995) and thus decrease the oxidative stress caused by NF- $\kappa$ B activation, a factor that has been found responsible for the coding of oxidative cytokines in most inflammatory conditions (Van Acker *et al.*, 1995). The reduction in acetic acid induced writhing and the increase in the reaction time to the thermal stimulus, suggest that the anti-nociceptive effect of *Tithonia diversifolia* are mediated both peripherally and centrally (Adeyemi *et al.*, 2002) since thermally induced painful stimuli are known to be selective to centrally but not peripherally acting analgesic drugs (Chau, 1989).

## CONCLUSION

In conclusion, this study has expressed scientific basis in support of the utilization of this plant in the traditional management of painful inflammatory conditions like arthritis.

Further studies are needed elucidate the exact mechanism of action of the extract of this plant as an anti-inflammatory and analgesic agent.

## REFERENCES

- Adeyemi, O.O., Okpo S.O., Ogunti O.O.** 2002: Analgesic and anti-inflammatory effects of aqueous extract of leaves of *Persia americana* Mill (Lauraceae). *Fitoterapia*. 73: 375-380.
- Alejandra, E.R., Teresita G.A., Osvaldo Juárez N.E., Lilian E.P.** 2003. Comparative study of flavonoids in experimental models of inflammation. *Pharmacology Research*48: 601-606
- Bork P.M., Schmitz M.L., Weidman K.M., Heinrich, M.** 1996. Ethno-botanical study of Anti-inflammatory and Antibacterial Activity in some selected plants. *Phytomedicine* 3: 263-269
- Chagas-paula D.A., Oliveira R.B., da Silva V.C., Gobbo-Neto L., Gasparoto T.H., Campanelli A..P. Faccioli L.H., Da Costa, F.B.** 2011. Chlorogenic acids from *Tithonia diversifolia* demonstrate better anti-inflammatory effect than indomethacin and its sesquiterpene lactones. *Journal of Ethnopharmacology* 136:355-62.
- Chau, T.** 1989: Pharmacology methods in the control of inflammation. (In) Modern methods in pharmacology Alan R Liss Inc., New York 4: 195-212.
- Cotran R.S., Kumar, V., Collins, T.** 2001. *Robbins pathological basis of disease 6th ed. W.B Saunder's company* 51.
- Cragg G.M., Newman, D.J.** 2003. Natural products in drug discovery and development. *Journal of Natural Products* 66: 1022-1037
- Crunkhon, P., Meacock, S.E.R.** 1971: Mediators of inflammation induced in the rat paw by carrageenan. *British Journal of Pharmacology* 42: 392-402.
- Denko, C.W.A.** 1992. Role of neuropeptides in inflammation. (In) Whicher, J.T., and Evans. S.W., *Biochemistry and inflammation Kluwer Publicity London*, 177-181.
- Diwan, P.V., Karwande, I., Margaret, I., Sattur P.B**1989: Pharmacology and Biochemical Evaluation of *Tridax Procumbens* on inflammation. *Indian Journal of Pharmacology* 21: 1-7.

- Goffin, E., Zierns, E., De Molps de., Madureira, M.C., Martins A.P., da Cunha A.P., Philippe G., Tits, M., Angenot, Angenit, L., Frederich, M.** 2002. In vitro anti-plasmodial activity of *Tithonia diversifolia* and identification of its main active constituent: Targitinin C. *Planta Medica*. 68 (6): 543-545.
- Hamowia, A.M., saffaf, A.M.** 1994, Pharmacological studies of *Vernonia amygdalina* (Del) and *Tithonia diversifolia* (Gray). *Veterinary Medical Journal*. 42:2, 91-97.
- Hang, V.T.H, Tuy C.D., Anh N.N., Preston, T.R.** 2012. Leaf extract from *Tithonia diversifolia* cures Scabies in rabbit. *Livestock Research for Rural Development*. 24: 1-8
- Hikino H.K., Ogata Y.K. and Konno C.**1985. Pharmacology of ephedroxanes. *Journal of Ethnopharmacology* 13: 175-191
- Jayawardena V.P., Rajaguru A.S.B. and Gunasena H.P.M.** 2000: Nutritive and Medicinal Values of *Erythrina. spp* and *Tithonia Spp.* *Proceedings of the Eleventh National Workshop on Multipurpose Trees*, Kandy, Sri-Lanka. 72-77.
- Katzung B.G.** 1998: Basic and Clinical Pharmacology. 7th ed. Stanford: Connecticut 578-579.
- Koster R., Anderson, M. De Beer E.J.** 1959. Acetic acid analgesic screening. *Federation Proceedings* 18: 412.
- Madureira, M.C., Martins, A.P., Gomes, M., Paiva, J., Cunha, A.P., Rosario V.** 2002: Antimalaria Activity of Medicinal plants used in traditional Medicine in Sao-Tome and Principe Islands. *Journal of Ethnopharmacology* 81: 23-29.
- Miura, T., Furuta, K., Yasuda, A., Iwamoto N., Kato, M., Ishihara, E., Ishida T., Tanigawa, K.** 2002. Antidiabetic Effects of Nitogbenku in KK-Ay *Diabetic mice*. *American Journal of Chinese Medicine* 30: 81-86
- Olukunle, J.O., Abatan, M.O., Adenubi, O.T., Oyebanji O.O., Ogbale O.O.** 2012. Anti-inflammatory and anti-nociceptive effects of extract and fractions of *Morinda morindoides* root. *International Research Journal of Pharmacy and Pharmacology*. 2(6): 118-125.
- Olukunle, J.O., Adenubi, O.T., Sogebi, E.A., Oguntoke, P.C** 2011. Analgesic and anti-inflammatory potential of *Jatropha carcus*. *Acta Veterinaria Brno* 80(3): 259-262.
- Owoyele, V.B., Wuraola, C.O., Soladoye, A.O., Olaleye, S.B.** 2003. Studies on the anti-inflammatory and analgesic properties of *Tithonia diversifolia* leaf extract. *Journal of Ethnopharmacology* 90: 317-321.
- Oyewole, I., Ibidapo, C., Moronkola, D., Oduola, A., Adeoye, G., Anyasor G.** 2008. Anti-malarial and antirepellent activities of *T. diversifolia* (Helmsl) *Journal of Medicinal Plant Research* 2: 171-175.
- Winter, C.A., Risley, E.A., Nuss, G.W.** 1962. Carrageenan induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proceeding of the Society for Experimental Biology and Medicine* (N.Y) 111, 207- 210
- Rainsford, K.D., Whitehouse, M.W.** 1980. Anti-inflammatory, anti-pyrexia salicylic acid esters with low gastric ulcerogenic activity. *Agents Action*. 10: 451-455.
- Uduak, E., Nodeley, Uriah,** 2013: Comparative phytochemical and physiochemical



- properties of *Aspilia Africana* and *Tithonia diversifolia* leaves. *International Journal of Modern Biology and Medicine*. 3:113-122
- Van Acker S.A., Tromp M.N., Haenen G.R., van der Vijgh W.J., Bast A.** 1995: Flavonoids as scavengers of nitric oxide radical. *Biochemistry, Biophysics Research Communication*. 214:755-9.
- Vinegar R., Screiber, W., Hugo, R.** 1969: Biphasic development of carrageenan oedema in rats. *Journal of Pharmacological Experiment and Therapeutics*. 166, 96-103
- Vohora, S.B., Khanna, T., Athar, M.** 1997. Analgesic activity of bacosine a new triterpene isolated from *Bacopa monnieri*. *Fitoterapia*. 64, 361-365.

(Manuscript received: 15th July, 2014; accepted: 19th February, 2015)