Analgesic, Anti-Inflammatory and Anti-Pyretic Activities Of *Tapinanthus Dodoneifolius (DC)* Danser Extract Using Several Experimental Models in Rodents.

*** Z. Abdullahi[1]; A. A. Jimoh[2]; E. B. Patrick[3]**
[1, 2, 3]Department of Pharmacology and Toxicology
Kaduna State University
Kaduna, Nigeria
*Corresponding Author: E-mail: zulaihatua@yahoo.com, [2]abdulfataijimoh@gmail.com
[3]patrickebadugu75@yahoo.com

Abstract
Tapinanthus dodoneifolius (TD) plant is used in ethno-medical practice by some tribes in northern part of Nigeria for the treatment of fever and pains. No scientific data confirming its use in the treatment of pain and inflammation has been reported. The aim of this study is to investigate and evaluate possible analgesic, anti-inflammatory and anti-pyretic activities of the methanol whole plant extract in rodents.
The anti-nociceptive activity study was carried out using three models of nociception in rats and mice (acetic acid-induced abdominal writhing, hot plate test, formalin-induced hind paw licking), while the effect of the extract on acute inflammation was carried out using Carrageenan-induced paw oedema in rats. Yeast-induced pyrexia model was used to evaluate possible anti-pyretic activity.
The extract produced a significant ($p < 0.01$) and dose-dependent inhibition of the acetic acid-induced abdominal constriction in mice and increased the threshold for pain perception dose-dependently in the hot plate test in mice. The extract also decreased both the acute and delayed phases of formalin-induced pain dose dependently ($p < 0.01$). The extract produced a significant ($p < 0.01$) dose-dependent anti-inflammatory effect in Carrageenan-induced oedema in rats.
These results suggest the presence of pharmacologically active constituents in the extract with nociceptive and anti-inflammatory activities that justifies its ethno-medical use in the management of pain and inflammatory conditions and subsequent development for clinical application.

Keywords: *Tapinanthus dodoneifolius; anti-nociceptive; anti-inflammatory; anti-pyretic activities; ethno-medicine.*

Introduction

In many communities in Africa, various treatment options are employed in the management of pain and inflammation, among which the use of herbal drugs is a very popular option. However scientific evaluation is needed to provide evidences of their safety and efficacy. In Sub-Saharan Africa, more than 80% of the population relies on traditional medicines and healers as the primary source of health care (WHO, 2002).This is mainly due to easy accessibility and affordability of consulting with traditional medical practitioners.
Tapinanthus dodoneifolius (TD) belongs to the family Loranthaceae. It is a bushy parasitic plant that grows on a wide range of trees and bushes of the wooded savannah zone. In Nigeria it is called kauchi (Hausa), Etu-lonchi (Nupe), Elozie (Igbo) and Afomu igba (Yoruba). The plant has been shown to have a wide range of activities, including anti-plasmodial activity

against *Plasmodium berghei* parasite, decrease in arterial blood pressure, for epilepsy, relaxant effect on trachea, antimicrobial activity against certain multiple resistant bacteria and fungal isolates and gynaecological disorders (Builders *et al*., 2012; Adewole, 2007; Ofem *et al*., 2007; Sylvin *et al*., 2005; Ouedraogio *et al*., 2005; Deeni and Sadiq, 2002). Other uses of the plant included for treatment of digestive disorders e.g. stomach ache, diarrhoea, dysentery; urinary tract infections and application on wounds. Cepeleanu *et al*. (1994) revealed larvicidal and molluscidal effects of *Tapinanthus dodoneifolius*.

The use of the plant in most of these conditions have been scientifically investigated and validated in studies that utilize various parts of the plant. To our knowledge there is no scientific report on its analgesic, anti-inflammatory and anti-pyretic properties. This study was therefore carried out to investigate the analgesic, anti-pyretic and anti-inflammatory activities of the whole plant extract in laboratory animals.

Materials and Methods

Collection and identification of plant material

Tapinanthus dodoneifolius whole plant was collected from Sabongari Local Government Area of Kaduna state. It was identified and authenticated at the Herbarium section in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. A voucher specimen (V/NO 350) was prepared and deposited at the herbarium for future references.

Plant preparation and extraction

The whole plant material was cleaned, air-dried in the shade and pulverized into coarse powder using mortar and pestle. The powder was stored in a dry air-tight container until it was used. Four hundred grams (400 g) of the coarse powdered plant was weighed and macerated in 2.5 L of 70% v/v methanol in water for 72 hours with constant shaking using a GFL shaker. The resultant mixture was filtered using muslin cloth, followed by Whatman filter paper (No.1). The filtrate was evaporated to dryness on a water bath. This gave a percentage yield of 39.7% w/w. Aliquot portion of the dried extract was weighed and dissolved in sterile distilled water for preparation of appropriate doses on each day of the experiment.

Drugs and reagents

Acetic acid (Searle Essex, England), formalin, Ketoprofen (Lek Pharmaceuticals company Ltd, Slovenia), 0.9% Normal saline, Distilled water, Brewer's yeast (Angel Yeast Company, China), Carrageenan and Morphine (Sigma-Aldrich chemical company, USA) were used in the study. Drugs were freshly prepared on the day of the experiments, and all treatments were injected via intraperitoneal (IP) or subcutaneous (SC) routes as indicated in each test. The extract doses were given orally.

Animals

Male and female Swiss albino mice (18-22g) and Wistar rats (160-200g) body weight obtained from the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used in the study. They were kept in clean, dry cages and maintained in well-ventilated animal house. Water and standard laboratory feed were given *ad libitum* for the duration of the study, except when fasting was necessary in the course of the study. All experiments conformed to the principles for research involving animals as recommended by the Principles of Laboratory Animal Care"(NIH Publication No. 85-23, revised in 1996) on the care and use of Laboratory animals.

Phytochemical screening

The methanol whole plant extract of *Tapinanthus dodoneifolius* was subjected to phytochemical screening for the presence of Phytochemicals according to methods described by Trease and Evans (1996) and Sofowora (2008).

Acute toxicity study

The modified method of Lorke (1983) was adopted. The study was carried out in two phases. In the first phase, 9 mice fasted overnight were randomly distributed into three groups of three mice each. Doses of 10, 100, 1000 mg plant extract/kg body weight were administered orally. This procedure was repeated using the intraperitoneal route. The mice were observed for signs of toxicity and mortality for the first critical four hours and thereafter daily for 7 days. Symptoms of all adverse effects and death were observed and recorded. The second phase was determined by the outcome of the first phase. Another set of three groups of three mice were given 1600, 2900 and 5000 mg extract/kg body weight orally. This procedure was repeated using the intraperitoneal route. These mice were also observed for signs of toxicity and pattern of mortality for the first four hours and thereafter daily for 7 days.

The LD_{50} was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1).

Assessment of anti-nociceptive and anti-inflammatory activity

Negative control animals received distilled water (10 mg/kg) and the positive control animals were administered the anti-nociceptive reference drug Ketoprofen (10 mg/kg) or morphine (4 mg/kg). The three extract treated groups received 100 mg, 200 mg and 400 mg/kg body weight respectively. Nociception was induced using the following experimental models:

Acetic acid-induced abdominal constrictions (writhing) test in mice

The abdominal constrictions resulting from intraperitoneal injection of 0.6% acetic acid consisting of the contraction of abdominal muscles together with the stretching of hind limbs, was carried out according to the procedure described by Correa *et al*. (1996). The mice were divided into five groups of six mice each. Group I mice served as the negative control and were administered 5 ml/kg body weight of normal saline. Mice in groups II, III and IV were given 100, 200 and 400 mg/kg body weight of the extract orally respectively. The group V mice received 10 mg/kg body weight Ketoprofen injection. Thirty minutes after treatment, all the mice were administered 0.6% v/v acetic acid at 10mg/kg body weight, ip. The numbers of writhing/constrictions were counted for 30 minutes, starting after 5 minutes of injection. Anti-nociceptive (analgesic) response was expressed as the reduction of the number of abdominal constrictions between control animals and mice pre-treated with the extract. The percentage inhibition was calculated.

Hot plate-induced pain test in mice

The effect of the extract on hot plate-induced pain in mice was used to measure the response latencies according to the method described by Turner (1963), and adopted by Vongtau *et al* (2004a). The hot plate was maintained at 55 ± 1^0 C and each mouse was placed into a glass beaker of 50 cm diameter on the heated surface. The time(s) taken to elicit nociceptive responses (after placement on hot plate) shown by lifting, shaking, sucking, licking of the paw or jumping off was recorded as the index of response latency. An automatic 30 second cut-off was used to prevent tissue damage in the absence of any response. Mice that showed these responses within 20 seconds were selected and randomly divided into five groups of six mice each, and were fasted for 24 hours but allowed access to water *ad libitum*. Group I mice (negative control) received 10 ml/kg of distilled water. Mice in groups II, III and IV were pre-treated one hour before with 100, 200 and 400 mg/kg extract orally respectively. Group V mice were injected with morphine 4 mg/kg one hour prior to their placement on the hot plate.

Formalin-induced hind-paw licking in rats

The procedure described by Dubuisson and Dennis (1977) as modified by Tjolsen *et al*. (1992) was adopted for the study. The amount of time spent licking the injected paw was timed and considered as an indication of pain. The responses were measured and recorded for 5 minutes after the Formalin injection (first neurogenic phase) and 15-30 minutes after Formalin injection (second inflammatory phase). Thirty albino male and female rats were

randomized into five groups of six rats each. The animals were fasted for 24 hours before the experiment, but were allowed access to water *ad libitum*. The rats in group I (negative control) received 10 ml/kg of distilled water; groups II – IV mice received 100, 200 and 400 mg/kg body weight of the extract orally respectively; while group V mice (positive control) received 4 mg/kg of morphine injection intraperitoneally. Thirty minutes after extract and drug administration, 0.05 ml solution of Formalin (2.5% Formaldehyde) was injected subcutaneously under the plantar surface of the left hind paw. The response for each rat was graded as follows:

0= Rat unaffected, stands or walk around on injected paw.
1= Injected paw favoured or partially lifted.
2= Complete lifting of injected paw.
3= licking, biting or chewing of injected paw.

Evaluation of anti-inflammatory activity

Thirty rats were randomly divided into 5 groups of 6 rats each. The hind paw size of each rat was measured using a digital Plethysmometer caliper (Precision measuring, China) at the start of the experiment. The rats in group I were administered 10 ml/kg distilled water intraperitioneally, while those in groups II, III and IV were injected intraperitioneally with 100, 200 and 400 mg/kg body weight extract respectively. The rats in group V were injected with 10 mg/kg body weight of Ketoprofen intraperitioneally. After the administration of extract, Ketoprofen and distilled water, the rats were allowed to rest for 30 minutes before 0.1ml of 1% Carrageenan was administered on the left hind paw, under the skin, between the fingers. First reading was taken after 1 hour, then thereafter hourly for 3 more hours. Increase in paw size (a measure of inflammation) was calculated as the difference between the paw size after Carrageenan injection and before the injection. Average values for each group was determined, for each hour.

Evaluation of anti-pyretic activity

The procedure described by Al-Ghamdi (2001), with slight modification, was adopted for the study. The body temperature of each albino Wistar rat was recorded by measuring the rectal temperature at pre-determined intervals of 1 hour. Fever was induced in the rats by injecting 15% w/v suspension of Brewer's yeast (*Saccharomyces cerevisiae*) at a dose of 10 ml/kg body weight subcutaneously. The rectal temperature of each rat was recorded after 24 hours of yeast administration by inserting digital thermometer (Omron Digital Fever thermometer, Omron® Healthcare, China) into the rectum of each rat. Rats that do not show a minimum increase of 0.5^0C in temperature 24 hours after yeast injection were discarded.

Twenty-five rats selected were grouped into five groups of five rats and were immediately treated as follows: group I received distilled water 10 mg/kg; while groups II, III and IV rats received 100, 200 and 400 mg/kg body weight of the extract respectively and group V rats 20 mg/kg of Paracetamol orally;. The temperature was again recorded for all the rats at 30, 60 and 90 minutes post treatment.

Statistical analysis

All quantitative data were expressed as the mean ± standard error of mean (SEM). Statistical analyses were carried out using one way analysis of variance (ANOVA). Anti-inflammatory evaluation data was analyzed using Mann-Whitney statistical test for non-continuous variables. Significant differences between means were assessed at 95% level i.e. $p< 0.05$ was considered significant.

Results

The percentage yield of the pasty dark green methanol whole plant extract was 39.7%. The preliminary phytochemical screening of MCETD revealed the presence of carbohydrate, cardiac glycosides, saponin glycoside, free anthraquinones, unsaturated steroid and triterpenes, tannins, and flavonoids. There were no alkaloids and combined anthracene.

The oral and intraperitoneal LD_{50} of the extract was found to be greater than 5000 mg/kg and 3800 mg/kg body weight respectively. The animals presented with paw- and genitalia-licking, salivation and calmness.

Treatment with MCETD at doses of 100, 200 and 400 mg/kg caused a significant ($p < 0.01$) and dose dependent reduction in the number of abdominal constrictions in mice when compared to the control group. Ketoprofen (10 mg/kg) had the highest reduction in the number of abdominal constrictions of all the doses used in the study, $p < 0.001$ (Table 1). All the extract doses administered significantly ($p < 0.05$) increased the latency to the onset of the first writhe observed in the control group.

Table 1: Effect of methanol whole plant extract of TD on acetic acid-induced abdominal constriction in mice

Treatment	No. of constrictions	% Inhibition
N/S 5 ml/kg	35.4 ± 2.302	
MCETD 100 mg/kg	17.6 ± 2.853*	50.3
MCETD 200 mg/kg	12.2 ± 1.718**	65.5
MCETD 400 mg/kg	11.4 ± 3.376**	67.8
Ketoprofen 10 mg/kg	9.60 ± 2.718**	72.9

MCETD = Methanol whole plant extract of *Tapinanthus dodoneifolius*; N/S = Normal saline

* $p < 0.01$;** $p < 0.001$; One way ANOVA; n = 6

In the hot plate test, MCETD at doses of 100, 200 and 400 mg/kg had significant ($p < 0.01$) dose-dependent analgesic activity when compared with the control group (Table 2). The 100, 200 and 400 mg/kg extract groups showed lowest pain threshold times at 60, 45 and 30 minutes after extract treatment (1.40, 2.51 and 3.15 seconds respectively). The higher doses of extract (200 and 400 mg/kg) significantly ($p < 0.01$) raised the pain threshold of the mice as indicated by the increase in reaction time. Morphine 4mg/kg had the highest analgesic activity of all the extract doses used in the study ($p < 0.001$). Morphine caused significant elevation of pain threshold that were more than those caused by the extract groups. The lowest pain threshold reaction time for morphine- treated group was obtained at 30 minutes after treatment (3.75 seconds) and the highest at 60 minutes (11.4. seconds).

Table 2: Effect of methanol whole plant extract of TD on analgesic activity in mice using the Hot Plate method

Treatment	Reaction time (seconds) after placement on hot plate			
	30 minutes	45 minutes	60 minutes	90 minutes
D/W 10 ml/kg	1.89 ± 0.098	0.99 ± 0.049	0.94 ± 0.0489	0.96 ± 0.065
MCETD 100mg/kg	1.72 ± 0.241	1.50 ± 0.216*	1.40 ± 0.188*	1.57 ± 0.184*
MCETD 200mg/kg	2.99 ± 0.269**	2.51 ± 0.404**	2.61 ± 0.102**	8.36± 4.331***
MCETD 400mg/kg	3.15 ± 0.425**	7.59 ± 3.608**	9.55±4.425***	8.18 ±3.016***
Morphine 4mg/kg	3.75 ± 1.278**	6.99 ± 1.776**	11.4 ±5.294***	4.79 ± 1.384**

MCETD = Methanol crude extract of *Tapinanthus dodoneifolius*; N/S= Normal saline

*$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; One way ANOVA; n = 6

In the Formalin hind paw test MCETD at doses of 100, 200 and 400 mg/kg body weight had significant ($p< 0.01$) dose dependent analgesic activity when compared to the control group (Figure 1). At the higher doses of 200 mg and 400 mg/kg, the extract showed significant inhibition of nociceptive responses in both phases respectively, dose and time-dependently in comparison with the control group. Morphine (4 mg/kg) showed the highest level of analgesia of all the doses used in the study ($p< 0.001$).

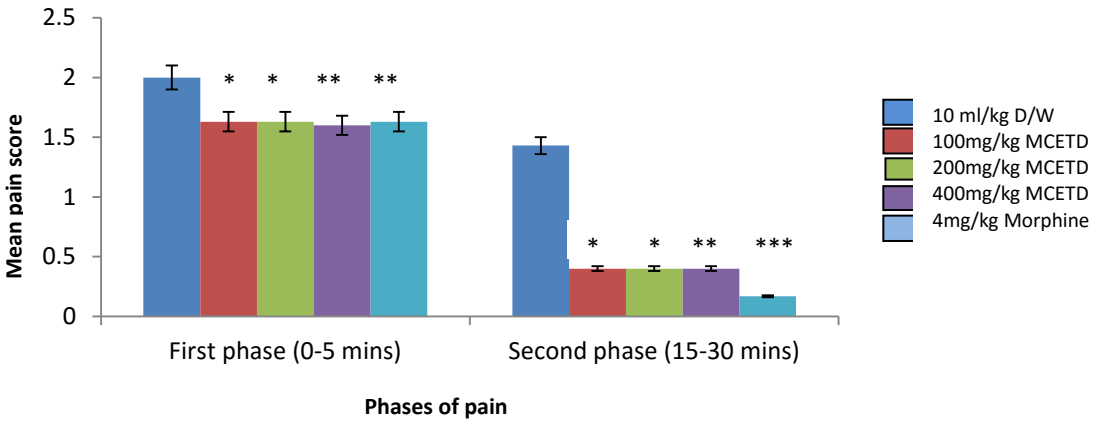

Figure 1: Effect of methanol whole plant extract of *Tapinanthus dodoneifolius* **on analgesic activity in rats using Formalin-induced hind paw licking**

*p < 0.05; **p < 0.01; ***p < 0.001; n = 6; MCETD = Methanol crude extract of *Tapinanthus dodoneifolius* D/W = Distilled water

MCETD at doses of 100, 200 and 400 mg/kg showed significant (p< 0.01 and p< 0.001) dose-dependent anti-inflammatory activity when compared with control untreated group. Highest activity was observed at 1 hour for the extract-treated groups. Ketoprofen had the highest activity at 2 hours, and its activity lasted for less than one hour, p < 0.01 (Figure 2). However, the 400 mg/kg extract-treated group showed activity that lasted for over 2 hours.

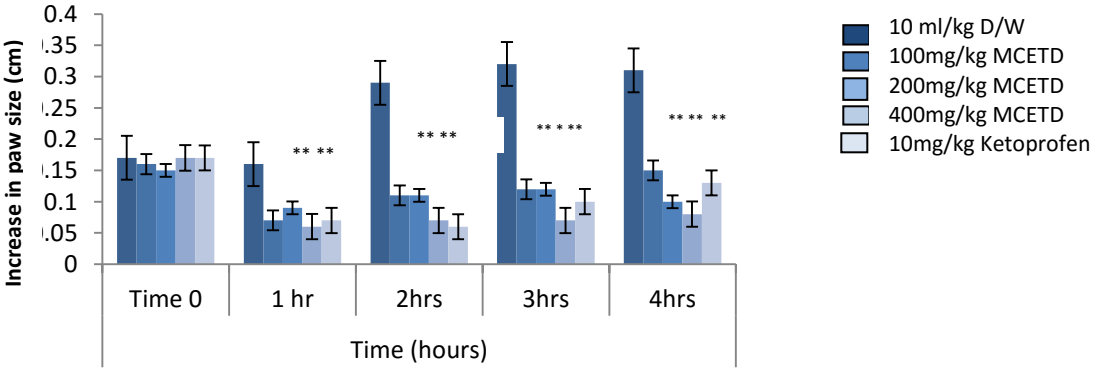

Figure 2: Effect of methanol whole plant extract of *Tapinanthus dodoneifolius* **on anti-inflammatory activity on rats**

MCETD = Methanol extract of *Tapinanthus dodoneifolius;* D/W = Distilled water* p < 0.01; ** p < 0.001 n =6;

MCETD significantly decreased the rectal temperature in rats dose-dependently, with the highest reduction occurring at dose of 400 mg/kg at 90 minutes. Paracetamol at a dose of 20 mg/kg produced the highest reduction in temperature compared to all the doses of the extract used in the test (Figure 3).

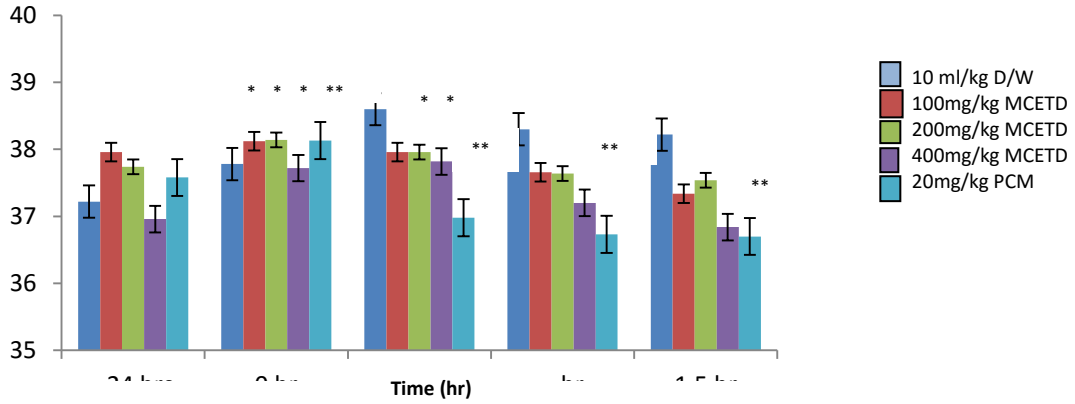

Figure 3: Effect of methanol whole plant extract of *Tapinanthus dodoneifolius on rectal temperature in rats*

D/W = Distilled water; MCETD = Methanol crude extract of *Tapinanthus dodoneifolius;* TD = *Tapinanthus dodoneifolius;* PCM = Paracetamol*;* * $p < 0.05$**;** ** $p < 0.01$**;** One way ANOVA; n = 5

Discussion

In the preliminary phytochemical screening, the presence of diverse constituents (secondary metabolites), offer support for the various ways in which this plant is used in traditional medicine, as these compounds are known to exhibit various physiological and biological activities *in-vivo* (Sofowora, 2008; Edeoga *et al*., 2005; Olajide, 2000).

The LD_{50} result suggested that acute oral administration of the extract was safe, and may also explain the reason why the whole plant extract is used in traditional treatment of many ailments.

The analgesic models were chosen to allow the investigation of both centrally and peripherally mediated anti-nociceptive activities, so as to allow the determination of the probable mechanism of effect of constituent of the extract. Analgesic activity of the extract may be peripherally mediated, centrally-mediated or both. Acetic acid abdominal constrictions method elucidates peripheral effects; hot plate test reveals central activity while formalin-induced nociception investigates both central and peripheral effects (Vongtau *et al*., 2004a).

Acetic acid is an irritating agent which stimulates local peritoneal receptors to induce pain with characteristic abdominal constrictions when injected into the peritoneal cavity. This effect has been associated with prostanoids in general, e.g. increased levels of PGE and PGF2α in the peritoneal fluids, as well as lipoxygenase products has been reported following the administration of acetic acid (Dhara *et al.,* 2000). The acetic acid-induced constriction test is very sensitive and is capable of detecting anti-nociceptive activity at a much lower dose level than are observable with other methods such as the tail-flick test (Bently et al., 1981). The inhibitory effect exhibited by the extract is suggestive of a peripherally mediated anti-nociceptive activity. This peripherally mediated action may be linked partly to inhibition of lipo-oxygenases and/or Cyclo-oxygenase. Diluted acetic acid administered to the peritoneal cavity induces viscera-motor responses acting indirectly by promoting the release of endogenous mediators which in turn stimulate the terminals of the primary afferent fibres, giving rise to the nociceptive feeling (Goettl and Larson, 1998).

The hot plate test, which utilizes thermal stimulus to induce pain, is frequently used to evaluate centrally mediated anti-nociceptive activity. The extract caused a considerable prolongation of reaction time to pain stimulus. This indicates an increase in the threshold for pain stimulus that suggests a central anti-nociceptive mechanism (Khanna *et al*., 1997). Centrally mediated action may likely be through opioid receptors in the CNS. Opioid analgesics relieve pain by raising the pain threshold at the spinal cord level and by altering the brain's perception of pain. The

analgesic property of the opioid, like morphine, are primarily mediated via μ receptors, however ƙ receptors in the spinal cord also contribute (Cherney, 1996). Morphine relieves both visceral and somatic pain without loss of consciousness. The ability of the crude extract to suppress pain perception in both the acetic acid-induced abdominal constriction and in the hot plate tests suggests that the nociceptive activity might be mediated via multiple mechanisms that involve both peripheral and central pathways of pain perception. Also glycosides, especially alcoholic glycosides, as was found to be present in MCETD, have been shown to have anti-pyretic, analgesic and anti-inflammatory activities.

The formalin test is a model for chronic pain that is useful in identifying both centrally acting anti-nociceptive agents such as narcotics, and peripherally acting analgesics such as acetyl salicylic acid (Vogel and Vogel, 1997; Elisabetsky *et al*., 1995). This test also distinguishes between non-inflammatory (early phase) and inflammatory (late phase) pain episodes according to the site and mechanism of action (Chan *et al*., 2000). Formalin induces pain based on two different processes by: stimulation of nociception in the paw that is centrally mediated (early phase); and secondly by activation of local inflammatory processes that stimulates pain sensation and to some degree, sensitization of nociceptive neurons (Tjolsen *et al*., 1992; Coderre and Melzack, 1992). Suppression of both phases of pain by the extract provides further evidence of dual activity involving both the centrally mediated and peripherally localized pain mechanisms.

Oedema induced by phlogistic agents is a widely accepted model for the evaluation of anti-inflammatory effect of drugs or chemical substances (Vongtau *et al*., 2004b). Previous studies have indicated the use of either egg albumin or Carrageenan as *in-vivo* models for acute inflammation to screen agents for anti-inflammatory activity. The Carrageenan-induced oedema results from the activation of the kinnin system, the accumulation of neutrophils and the release of several inflammatory mediators such as prostanoids and cytokines. Mast cell amines play a minor role in this inflammatory reaction. The primary mechanism of action of Ketoprofen, the standard drug used, is inhibition of prostaglandin synthesis by interfering with the Cyclo-oxygenase pathway of arachidonic acid metabolism. The extract showed a dose-dependent suppression of the inflammation induced by Carrageenan, which is highly suggestive of the presence of an anti-inflammatory activity.

Also, flavonoids (found to be present in MCETD) have been reported to be involved in anti-inflammatory activity of plants. Flavonoids are well known effective natural anti-inflammatory agents (Ueda *et al*., 2004) that can also produce antispasmodic effect (Yonathan *et al.*, 2006). The extract may thus be acting through a combination of these mechanisms to produce its anti-inflammatory effect. Also tannins, present in MCETD extract, have been known to have astringent property and are used for inflamed mucus membrane. Constituents such as triterpenes and Saponins are known to exert diverse pharmacological actions such as analgesic, central nervous system and cardio-vascular effects (Hussain and Deeni (1991). The analgesic and anti-inflammatory effects of the extract may therefore be due to the presence of bioactive Phytochemical such as Tannins, flavonoids and Saponins that were present in the extract.

The yeast-induced pyrexia has been used by various workers for screening of agents for acute anti-pyretic activity (Adzu *et al*., 2003). The yeast induces pyrexia by increasing the synthesis of prostaglandins. The anti-pyretic activity models are used to screen new substances for anti-pyretic activity. It is well-known that most anti-inflammatory analgesic drugs or substances also possess anti-pyretic activity as well. In general, non-steroidal anti-inflammatory drugs produce their anti-pyretic action through inhibition of prostaglandin synthesis within the hypothalamus (Hajare *et al*., 2000). The presence of flavonoids compounds in the extract may be responsible for its anti-pyretic activity, as flavonoids are predominant inhibitors of Cyclo-oxygenase or Lipoxygenase.

Conclusion

These results suggest that the methanol whole plant extract of *Tapinanthus dodoneifolius* may contain bioactive constituents with analgesic, anti-inflammatory and anti-pyretic activities, and further supports the ethno-medical use of the plant in the management of pain and inflammatory conditions.

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References

Adewole, SO; Ojewole, JAO. (2007). Hypoglycemic and hypotensive effects of *Tapinanthus dodoneifolius* leaves. *Cardiovascular Journal of South Africa*.

Adzu, B.; Abbah, J.; Vongtau, H.; Gamaniel, K. (2003). Studies on the use of *Cassia singgueana* in malaria Ethnopharmacy. *Journal of Ethnopharmacology* 88, 261-267.

Al-Ghamdi, MS. (2001). The anti-inflammatory, analgesic and anti-pyretic activity of *Nigella sativa*. *Journal of Ethnopharmacology* 76(1): 45-48.

Bently, GA; Newton, SH and Starr, J. (1981). Evidence for an action of morphine and enkephalines on sensory nerve endings in the mouse peritoneum. *British Journal of Pharmacology* 73: 325-332.

Builders, MI; Oguru, MO; Aguiyi, C. 2012. Anti-plasmodial potential of the African mistletoe: *Agelanthus dodoneifolius* Polh and Wiens. *Indian Journal of Pharmaceutical Sciences*. Volume 74(3), 223-229.

Cepeleanu, F., Hamburger, MO., Sordat, B. (1994). Screening of tropical medicinal plants formolluscidal, larvicidal, fungicidal and cytotoxic activities and brine shrimp toxicity. *International Journal of Pharmacognosy*. 32: 294-307.

Chan, K; Islam, MW; Kamil, M; Radhakrishna, R; Zakaria, MNM; Habibullah, M and Attas, A. (2000). The analgesic and anti-inflammatory effects of *Portulaca oleracea L. Subsp.* Sativa (Haw). Celak. *Journal of Ethnopharmacology* 773: 445-451.

Coderre, TJ., Vacarine, AL., Melzack, R. (1992). Central nervous system plasticity in the tonic pain response to subcutaneous formalin injection. *Brain Research*. 535: 155 - 158.

Deeni, YY.; Sadiq, NM.(2002). Anti-microbial Properties and Phytochemical constituents of *Tapinanthus dodoneifolius* (DC) Danser (Loranthaceae): An Ethno medicinal Plant of Hausa land, Northern Nigeria.

Dharra, AK.; Suba, V.; Sen, T.; Pal, S.,Nag Chaudhuri, AK. (2000). Preliminary studies of the anti-inflammatory and analgesic activity of the methanolic fraction of the root of *Tragia involucrate*. *Journal of Ethnopharmacology* 72, 265-268.

Edeoga, HO., Okwu, E. and Mbaebie, BO. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*; 4(7): 685-688.

Elisabetsky, E., Ahmador, TA., Albuquerque, RR., Nunes, DS., Caravalho, ACT. (1995). Analgesic activity of *Pyschotria colorata* (Wild. Ex-R and S) Muell. Arg. Alkaloids. *Journal of Ethnopharmacology*. 48: 77-83.

Goettl, VM., Larson, AA. (1998). An antagonist of substance P N-terminal fragments, D substance P(1-7) reveals that both nociceptive and anti-nociceptive effects are induced by substance P N-terminal activity during noxious chemical stimulation. *Brain Research* 780:80-85.

Hajare, SW., Chandra, S., Tondon, SK., Sharma, J., Lal, J., Telang, AG. (2000). Analgesic and anti-pyretic activities of *Delbergia sissoo* leaves. *Indian Journal of Pharmacology*. 32: 357-360.

Hussain, HSN., Deeni, YY. (1991). Plants in Kano Ethno-medicine: Screening for

antimicrobial activity and alkaloids. *Indian Journal of Pharmacology*. 29, 51-56.

Khanna, AT.; Sivaramann, R., Vohora, SB. (1997). Analgesic activity of silver preparations used in Indian systems of medicine. *Indian Journal of Pharmacology*. 29, 393-398.

Ofem, OE; Eno, AE; Imoru, J; Nkanu, E; Unoh, F.; Ibu, JO. (2007). Effect of the aqueous leaf extract of *Tapinanthus dodoneifolius* on arterial blood pressure and heart rate in Wistar rats under Pentobarbitone anaesthesia. *Indian Journal of Pharmacology*. Vol. 39, Issue 1, Pg 15-19.

Olajide, OO; Awe, SO; Makinde, M. (2000). Studies on the anti-inflammatory, anti-pytrtic and analgesic properties of *Atstonia boonei* stem bark. *Journal of Ethnopharmacology*. 71: 197-186.

Ouedraogo, M.(2005). Relaxant effects of hydro-alcoholic extract of *Tapinanthus dodoneifolius* on Wistar rat trachea *in-vitro. African Journal of Traditional, complementary and alternative medicine*. Vol.2: 2, Pg 166-176.

Sofowora, A. (2008). *Medicinal plants and Traditional medicine in Africa*. 3rd Edition Spectrum books Ltd. Ibadan, Nigeria. 199-202.

Sylvin, O. (2005). Cardiovascular Properties of aqueous extract of *Tapinanthus dodoneifolius* on isolated rat aorta and heart. *African J. Traditional, Complimentary and alternative medicine*. 2 (1): 25-30.

Tjolsen, A.; Berge, OG.; Hunskaar, S.; Rosland, JH., Hole K. (1992). The formalin test: An evaluation of the method. *Pain* 51, 5-17.

Ueda, H., Yamazaki, C., Yamazaki, M., (2004). A hydroxyl group of flavonoids affects oral anti-inflammatory activity and inhibition of systemic tumour necrosis factor-alpha production. *Bioscience, Biotechnology and Biochemistry* 68, 119–125.

Vogel, HG., Vogel, WH. (Eds). (1997). Drug discovery and Evaluation: *Pharmacological Assays*. Springer-Verlaag. Berlin. Heldelberg.

Vongtau, HO., Abbah, J., Masugu, O., Chindo, BA., Ngala, IE., Salawu, AO., Kwanashie, HO., Gamaniel, KS. (2004a). Anti-nociceptive profile of the methanolic extract of *Neurautanenia mitis* root in rats and mice. *Journal of Ethnopharmacology* 92: 317-324.

Vongtau, HO.; Abbah, J.; Ngazal, IE.; Kunle, OF.; Chindo, BA.; Otsapa, PB.,Gamaniel, KS. (2004b). Anti-nociceptive and anti-inflammatory activities of the methanolic extract of *Parinari polyandra* stem bark in rats and mice. *Journal of Ethnopharmacology*. 90, 115-121.

World Health Organization (WHO). (2002). *WHO fact sheet* number 271. WHO, Geneva.

Yonathan, M., Asres, K., Assefa, A., Bucar,F., (2006). *In-vivo* anti-inflammatory and anti nociceptive activities of *Cheilanthes farinosa*. *Journal of Ethnopharmacology* 108, 462–470.

TITLES AND LEGENDS TO FIGURES