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Full Length Research Paper

Antinociceptive and Anti-Inflammatory Activities of Methanol Root Extract of *Tetracera potatoria*

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ABSTRACT

Methanol root extract of *Tetracera potatoria* was evaluated for anti-inflammatory and analgesic activities. The inflammatory activity of the extract was assayed by egg-albumen induced paw oedema and formalin paw lick test in rats whereas its analgesic activity was determined by its effects on acetic acid-induced abdominal writhing, hot plate and tail flick tests in mice. Each of the experiments consisted of twenty five animals randomly, but equally divided into 5 groups of 100, 300 or 500 (mg kg^{-1}) body weight extract pre-treated, Indomethacin pre-treated (10 mg kg^{-1} b.w) and a control group administered with distilled water (10 mL Kg^{-1} b.w). At the 30, 60 and 90 minutes post-injection of albumen observation periods, there was significant ($p<0.05$) inhibition of egg-albumen induced paw oedema in rats pre-treated with extract of *T. potatoria* especially 500mg/kg b.w (69.4, 64 and 76%) compared with rats in the control group and Indomethacin-pretreated rats (18.4, 24 and 32%). Itching of the paws persisted significantly ($p<0.05$) longer in the control or Indomethacin-treated rats than in *T. potatoria* pre-treated for both phases of formalin-induced paw licks. Extract-treated animals exhibited significantly ($p<0.05$) lower number of writhings in response to acetic acid induced writhing movement. The analgesic effect of the extract (100, 300 or 500mg kg^{-1}) in hot plate test was observable within 60 minutes of administration and maximum effect obtainable 120 minutes post-administration, just as pre-treated mice tolerated thermal pain longer than the Indomethacin-pretreated counterpart in tail flick test. Findings from this study showed that extract of *T. potatoria* possess anti-inflammatory and analgesic potencies most probably via the central and peripheral mechanisms. Isolation and chemical characterization of the bioactive compound in this plant is warranted and therefore recommended as further studies..

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Keywords*:* *Tetracera potatoria*, anti-inflammatory, analgesic, mice, rats

INTRODUCTION

Inflammation is generally defined as the response of living tissues to an injurious stimulus and is recognised as a primary defense mechanism (Abbas and Lichman, 2009). The magnitude of the inflammatory response is important because insufficient responses result in immunodeficiency, while excessive responses are associated with diseases such as rheumatoid arthritis, Crohn's disease, atherosclerosis, diabetes, Alzheimer's disease, multiple sclerosis, and cerebral and myocardial ischaemia (Tracey, 2002). Septic shock syndrome, sepsis, meningitis and severe trauma occurs as a result

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of spread of inflammation into the blood stream and the inflammatory responses can be more exaggerated than the response at the original inciting stimulus (Tracey, 2000). Certain drugs or chemical compounds are known to control inflammation. Some plants have also been reported to possess anti-inflammatory activity and these include *Sambucus ebulus* rhizome (Ahmadiani *et al.*, 1998) *Vitis trifolia, Caralluma tuberculata* (Ahmed *et al.*, 2007), *Morinda lucida* (Awe *et al.*, 1998), *Combretum micranthum, Bridelia ferruginea, Alstonia boonei* (Olajide *et al.*, 2000a), *Asparagus africanus* (Hassan *et al.*, 2008) *Chlorella stigmaphora* and *Phaeodactylum tricormutrum* (Guzman *et al.*, 2001), *Careya arborea* (Gupta *et al.*, 2006) *Sida acuta* (Oboh and Onwukaema, 2005), *Tithonia diversifolia* (Owoyele *et al.*, 2004) to mention a few.

Tetracera potatoria (family Dilleniaceae) is one of the several other plants used traditionally for treatment of diseases of inflammatory origin which are yet to be scientifically verified. It is a scandent shrub or climber up to 5 m long. The stem contains a clear watery sap which could be obtained by cutting (Oluwole *et al.*, 2008). The plant is widespread from Sudan and Congo Republics into West Africa. The sap and the powdered leaf are used in Nigeria, West Africa for the treatment of toothache and cough, while the aqueous extract of the root is an active remedy for intestinal disorders (Adekunle *et al.*, 2003). Root decoction of the plant is used to treat gonorrhoea and other veneral diseases in Sierra-Leone ethnomedical practice (Burkill, 1985).

The current study is aimed at evaluating the anti-inflammatory and analgesic activity of *T. procera* with probable understanding of the underlying mechanism which justifies the traditional uses of the plant.

MATERIALS AND METHODS

Preparation of plant extract: Roots of *Tetracera potatoria* were harvested and identified at the herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife by Mr. Ademoriyo with voucher specimen number 16425. The roots of *T. potatoria* were dried in the oven at 40ºC for 1 week, after which they were pulverized using the hammer mill. 500g of the powder was macerated in methanol for 72 hours. The resulting extract was filtered and concentrated in rotary evaporator at 35ºC, after which it was freeze dried to give the solid extract. A yield of 90g (18%) was obtained.

Experimental Animals: Swiss mice (18-22g) and Wistar rats (150-180g) were used for the study. The animals were housed at the Animal House, Obafemi Awolowo University, Ile-Ife, Nigeria and maintained under standard environmental conditions. They were allowed access to standard rat diet and water *ad libitum*. The animals were divided into 5 groups of five animals for each of the experiments. The first three groups were administered with the extract doses at 100, 300 and 500mg/kg body weight (b.w.). Group 4 was administered with indomethacin (10mg/kg b.w), while group 5 received distilled water (10ml/kg) only. The Animal Ethical Committee of the Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife approved the protocols of the study.

Anti-inflammatory Study

Egg albumen induced rat paw oedema: Rats used for this study were fasted overnight and the experiment was conducted according to the method described by Okoli *et al.* (2007). The rats were grouped as described above into three groups which were administered with the extracts, the 4th group which served as the positive control and the 5th group serving as negative control. One hour after administration of the extract or indomethacin, pedal oedema was induced by injection of 0.2ml (2% w/v) egg albumen into the right hind paw of each rat under the sub plantar region. The paw sizes were measured by using the cotton thread method (Hess and Milonig, 1972; Olajide *et al.*, 2000b) before injection of albumen and immediately (0hr) after the injection. Subsequent measurements were done at 0.5, 1, 1.5 and 2 hours post injection of albumen. Cotton thread was wrapped around the paw and the circumference was measured with a meter rule. The inhibitory activity was calculated according to the following formula;

$$\text{Inhibition (\%)} = \frac{(C_1 - C_0)\ \text{control} - (C_1 - C_0)\ \text{treated}}{(C_1 - C_0)\ \text{control}} \times 100$$

Where C_1 is paw size after albumen injection, C_0 is paw size before albumen injection

Formalin-induced paw lick test in rats: Rats in this experiment were groups as mentioned above. 20µL of 2.5% formalin was injected into the plantar surface of the left hind-paw of the rat (Tjolsen *et al.*, 1992). One hour post-administration of the root extract (100, 300 and 500mg/kg, p.o.), indomethacin (10mg/kg b.w) or distilled water (10mls/kg b.w), The amount of time spent licking the injected paw was indicative of pain. The number of licking within 0 to 5 minutes (first phase) and 20–30 minutes post-injection of formalin (second phase) were counted.

Analgesic Study

Acetic acid-induced abdominal writhing test in mice: Mice used in this experiment were also grouped as mentioned above. The mice were administered with fruit extract (100, 300 and 500mg/kg, p.o.), aspirin (10mg/kg b.w) or distilled water (10mls/kg b.w). One hour post-administration of the extract or drug, the mice were injected intraperitonealy with 0.1ml/10g body weight of 0.6% acetic acid solution to induce the characteristic writhing. The numbers of writhing were observed between 5-15 minutes post-administration of acetic acid.

Hot plate test in mice: Pain reflexes in response to a thermal stimulus were measured using a hot plate apparatus (Ugo Basile, Italy). Mice were habituated to the apparatus for 1min before the start of the test. They were placed on the hot plate of 25.4cm x 25.4cm at $(55\pm1.0)^0$C, which is surrounded by an opened-top acrylic cage (19cm tall), with the start/stop button on the timer. A 10 seconds cut-off time was used to prevent tissue damage. The latency measures were taken both before, 60 and 120 minutes after drug/extract administration as the time elapsed between placing the mice on the hot plate and fore paw licking, hind paw flicking or jumping. The mouse was immediately removed from the hot plate and returned to its home cage.

Tail flick test: This experiment was conducted according to the modified method adopted by Sanchez-Mateo *et al*. (2006) using hot water bath. Groups of five mice each were administered with the root extract, indomethacin or distilled water respectively as earlier mentioned. At 0, 60 or 120 minutes post-treatment, the terminal 2 cm of each mouse tail were immersed in water bath containing hot water maintained at 55±1°C by a circutine (Haake-Vison, Germany). The time taken in seconds taken by the mice before the first response to pain was recorded.

Statistical analysis

Data were analysed using one way analysis of variance (ANOVA) on GraphPad Prism 4.0 version. The result obtained were expressed as mean values ± standard error of mean (SEM). The statistical significant difference between the mean values were determined at $p<0.05$.

RESULTS

Anti-inflammatory Study

The inflammatory responses of rats administered with the root extract were reduced compared to rats pre-treated with indomethacin or distilled water. The rats administered with extract of *T. potatoria* dose-dependently inhibited the paw oedema induced by the egg albumen. The inflammatory response in paw of rats administered with the extract at doses of 300mg/kg (0.74±0.10 and 0.74±0.05 cm) and 500mg/kg b.w (30±0.10 and 0.36±0.22 cm) were reduced compared to that observed in rats administered with indomethacin (10mg/kg) at 30 and 60 minutes post-injection (0.80±0.05 and 0.76±0.07 cm), while all extract doses inhibited formation of oedema more significantly ($p<0.05$) at 90 and 120 minutes post-injection (Table 1). Pre-treated rats endured pain at the early and late phases of formalin-induced paw lick for shorter period at extract dose of 100mg/kg b.w (38.50±4.60 and 0.50±0.50 seconds) compared with those administered with 500mg/kg b.w dose of the extract (9.5±4.3 and 0.0±0.0 seconds). Rats administered with the three doses of the extract tolerated pain for longer compared to rats administered with indomethacin (42.00±3.10 and 7.00±4.60 seconds) (Table 2).

Table 1:
The effect of *Tetracera potatoria* methanol extract on change in paw sizes (cm) and percentage inhibition of oedema in rat injected with egg albumen

Group	30mins	60mins	90mins	120mins
Extract (100mg/kg)	0.86 ± 0.10^{b} (12.2%)	0.92 ± 0.04^{a} (8%)	0.36 ± 0.05^{b} (67%)	0.32 ± 0.07^{cb} (71%)
Extract (300mg/kg)	0.74 ± 0.10^{ab} (24.9%)	0.74 ± 0.05^{a} (26%)	0.36 ± 0.04^{b} (67%)	0.26 ± 0.05^{c} (76%)
Extract (500mg/kg)	0.30 ± 0.10^{a} (69.4%)	0.36 ± 0.22^{b} (64%)	0.26 ± 0.13^{b} (76%)	0.20 ± 0.11^{c} (81.8%)
Indomethacin (10mg/kg)	0.80 ± 0.05^{ab} (18.4%)	0.76 ± 0.07^{a} (24%)	0.76 ± 0.10^{a} (32%)	0.50 ± 0.10^{b} (55%)
Control	0.98 ± 0.02^{b} (0%)	1.00 ± 0.07^{a} (0%)	1.10 ± 0.07^{a} (0%)	1.10 ± 0.08^{a} (0%)

Values with different superscript are statistically significant at P<0.05

Table 2:
Response (in seconds) of rats administered with *Tetracera potatoria* methanol extract to formalin paw lick test

Group	Early phase	Late phase
Extract (100mg/kg)	38.50±4.60[a]	0.50±0.50[a]
Extract (300mg/kg)	56.00± 4.00	2.50±2.50[b]
Extract (500mg/kg)	9.50±4.30[b]	0.00± 0.00[c]
Indomethacin (10mg/kg)	42.00±3.10[c]	7.00±4.60[d]
Control	64.50±6.70[d]	65.00± 2.10[e]

Values with different superscript in a column are statistically significant at p<0.05

Table 3:
The effect of acetic acid on abdominal writhing in mice administered with methanol extract of *Tetracera potatoria*

Treatment	No of writhing
Extract (100mg/kg)	30.4 ± 5.4[a]
Extract (300mg/kg)	26.6 ± 7.5[ab]
Extract (500mg/kg)	15.0 ± 4.4[b]
Indomethacin (10mg/kg)	16.4 ± 2.7[b]
Control	35.2 ± 1.2[a]

Values with different superscript are statistically significant at p<0.05

Analgesic Study

The mice administered with the extract showed reduced abdominal writhing (30.4±5.4, 26.6±7.5 and 15.0±4.4 abdominal writhes) in a dose-dependent pattern to pain induced by intraperitoneal injection of acetic acid. Rats administered with the extract at the dose of 500mg/kg b.w showed lesser abdominal writhing compared to rats administered with indomethacin (16.4±2.7 abdominal writhes) (Table 3). The mice administered with the extract demonstrated significant (p<0.05) and dose-dependent tolerance to thermal pain in both the hotplate and tail flick tests at 60 and 120 minutes post-administration. In the hot plate test, rats administered with the extract at the dose of 500mg/kg b.w (3.96±0.24 seconds) showed lesser tolerance to pain at 60 minutes post-administration compared with indomethacin (4.06±1.10 seconds). At 120 minutes post-administration, all three doses (3.90±0.08, 3.96±0.08 and 4.80±0.21 seconds) significantly (p<0.05) inhibited pain sensation more than indomethacin (3.27±0.46) (Table 4).

The tail flick test on the other hand demonstrated that mice administered with the extract were more tolerant to thermal pain at 60 minutes (5.22±1.08, 5.87±1.58 and 7.38±1.18 seconds) and 120 minutes (5.55±1.56, 7.37±2.34 and 9.43±2.66 seconds) post-administration compared with mice administered with indomethacin (4.00±0.56 and 5.10±0.78 seconds) (Table 5).

DISCUSSION

In this study, the methanol extract of the root of *Tetracera potatoria* demonstrated potent anti-inflammatory and analgesic activities in a dose-dependent pattern. The extract at the doses of 100, 300 and 500mg/kg b.w administered to the rats or mice exhibited more potent anti-inflammatory or analgesic activity compared to indomethacin (10mg/kg b.w). The extract significantly (p<0.05) inhibited formation of oedema and induction of inflammatory pain in a dose dependent manner in rats administered with the extracts.

Table 4:
The effect of *Tetracera potatoria* methanol extract on tolerance (in seconds) to thermal pain induced by hot plate method in mice

Group	0 minute	60 minutes	120 minutes
Extract (100mg/kg)	3.3 ± 0.03[a]	3.70 ± 0.50[a]	3.90 ± 0.08[b]
Extract (300mg/kg)	3.3 ± 0.03[a]	3.72 ± 0.39[a]	3.96 ±0.08[b]
Extract (500mg/kg)	3.33 ±0.04[a]	3.96 ±0.24[a]	4.80 ±0.21[a]
Indomethacin (10mg/kg)	3.33±0.9[a]	4.06±1.1[a]	3.27±0.46[c]
Control	3.6 ±0.52[a]	2.89 ±0.16[b]	1.92 ±0.3[d]

Values with different superscript are statistically significant at P<0.05

Table 5:
The effect of *Tetracera potatoria* methanol extract on tolerance (in seconds) to thermal pain induced by tail flick method in mice

Group	0 minute	60 minutes	120 minutes
Extract (100mg/kg)	4.24±0.88[a]	5.22±1.08[abc]	5.55±1.56[ab]
Extract (300mg/kg)	5.90±1.46[a]	5.87±1.58[abc]	7.37±2.34[ab]
Extract (500mg/kg)	5.25±1.47[a]	7.38±1.18[a]	9.43±2.66[a]
Indomethacin (10mg/kg)	5.33±0.93[a]	4.00±0.56[bc]	5.1±0.78[ab]
Control	5.00±0.20[b]	1.80±0.30[c]	2.0±0.26[b]

Values with different superscript are statistically significant at P<0.05

Oedema is attributed to the synthesis of mediators of acute inflammation such as histamine, serotonin and prostaglandin and ability of any agents to inhibit oedemagenic agents is traced to inhibition of these mediators of acute inflammation (Olaleye *et al.* (2004). Inhibition of oedema by *T. Potatoria* in this study could therefore be explained by its ability to inhibit synthesis of these mediators of inflammation.

The early (neurogenic) and late phase responses of rats to inflammatory pain in the formalin paw lick model presents a more vivid explanation of the two phases of inflammation and the mechanisms involved. The two phases of response are usually due to direct stimulation of nociceptors in the paw which culminates in centrally mediated inflammatory pain with release of substance P in the neurogenic (early) phase. The late phase on the other hand is observed as a result of release of histamine, serotonin, bradykinin and prostaglandins (Zeashana *et al*., 2009). Indomethacin, a well known NSAID inhibits only the late phase of this model, while centrally acting anti-inflammatory agents inhibit both phases (Stai *et al*., 1995). *T. potatoria* extract exhibited a significantly potent inhibition of inflammatory pain at both early and late phases. The late phase response was totally abolished by the extract at 500mg/kg b.w. Inhibition of both early and late phase responses is an indication that *T. potatoria* possess centrally- and peripherally-mediated anti-inflammatory properties.

Mice administered with the extract demonstrated longer tolerance to pain induced by acetic acid. The number of abdominal writhing observed in these mice, especially those administered with the extract at 500mg/kg b.w were lesser than that observed for mice administered with Indomethacin (10ml/kg b.w). Even though, the acetic acid-induced abdominal writhing test exhibited the analgesic activity of *T. potatoria*, the model itself is non-specific because it does not indicate whether the analgesic effect is mediated centrally or peripherally (Magaji *et al*., 2008). The thermal tests (hot plate and tail flick tests) strongly indicate the mechanism involved in the anti-nociceptive pathway. Pain is mediated both peripherally and/ or centrally, with different mechanisms responsible for both phases. Peripheral analgesic effect is mediated via inhibition of cycloxygenases and/or lipoxygenases (and other inflammatory mediators), while central analgesic effect is mediated via interactions with adrenergic, serotonergic, cholinergic and dopaminergic receptors (Garcia *et al*., 2004). The hot plate test is effective in determining centrally acting analgesics by their ability to increase the time of response (García, *et al.*, 2004). The tail flick test on the other hand is used to determine both centrally acting analgesics (Ramabadran *et al*., 1989) such as morphine (Domer, 1990), and peripherally acting analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs). Mice administered with *T. potatoria* in this study were more tolerant to thermal pain induced by both hot plate and tail flick tests within 60 minutes of administration and the longest tolerance to pain attained at 120 minutes post-administration of the extract, thus signifying both peripheral and/or central mediated analgesia.

In conclusion, this study validates the traditional indication for use of the roots of *T. potatoria* as an anti-inflammatory and analgesic herb. The root extract is also suggested to act via both centrally and peripherally mediated mechanisms of anti-inflammation and analgesia. Further studies are imperative to isolate and chemically elucidate the active principle(s) responsible for these bioactivities. This may lead to development of new anti-inflammatory and analgesic drugs from our natural environment which will be more ecologically friendly and environmentally tolerant.

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