Original article

Phytochemical constituents, analgesic and anti-inflammatory effects of methanol extract of *Triumfetta rhomboidea* leaves in animal models

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Abstract

Objective: To investigate the analgesic and anti-inflammatory effects of the methanolic extract of the leaves of *Triumfetta rhomboidea* on mice and rats respectively. And to screen the phytochemical constituent of the extract. **Methods**: The analgesic effect was determined by acetic acid-induced writhing test in mice. While the anti-inflammatory activity was determined by egg albumin-induced oedema of the rat paw. Phytochemical screening was done by standard procedures. **Results**: *Triumfetta rhomboidea* leaf extract (50 – 400 mg/kg) caused a statistically significant inhibition on the egg albumin-induced eodema or inflammation in Wister albino rats with $P < 0.001$ (ANOVA). This effect was higher than the observed effect with Piroxicam (0.5 mg/kg) which was used as a standard. The effect was also dose-dependent. Furthermore, *Triumfetta rhomboidea* extract caused a statistically significant reduction in the number of acetic acid-induced writhing in mice, with $P < 0.001$ (ANOVA). These effects were also does-dependent and greater than the analgesic effects by paracetamol which was used as a reference drug. Phytochemical screening revealed the presence of flavonoids, steroids, triterpenoids alkaloids, tannins and saponins in *Truimfetta rhomboidea* leaf extract. **Conclusion**: *Triumfetta rhomboidea* can be recommended for acute inflammatory disorders and diseases associated with pains. This also supports its traditional use as an anti-snake bite and anti-cancer or anti-tumor agent. Further study is on the way to find out the mechanism of its action and also to isolate, identify and characterize the active principle responsible for these effects in this plant.

Keywords: *Triumfetta rhomboidea*; Phytochemical constituents; Anti-inflammatory; Analgesic effect; Animal models

INTRODUCTION

Triumfetta rhomboidea Jacq. (Family: Tiliacea) is commonly known as Burr bush/Burweed or Chinese Burr (English), herbe apanier (French). Locally, in Nigeria, it is called Udo (Igbo), llasa omode or akabolo-bolo (Yoruba).

The plant is a weedy under-shrub, with variable habitant and foliage, occurring almost everywhere across the whole region, and widely distributed in tropical and subtropical India, Ceylon, Malay Peninsula, China, and Africa and in America[1, 2].

Tiumfetta rhomboidea (*T. rhomboidea*) Jacq is used traditionally in Rwanda as abortifacient, treatment of snake-bite [3]. In Ayurveda, the root is used as bitter, acrid, aphrodiasiac, tonic, cooling and in dysentery [2]. The leaves and stem are used as a poultice on tumors [2].

Powdered leaf infusions of *T. rhomboidea* are used for the treatment of anaemia in various regions of East Africa[4]. In Folklore remedy, the plant was

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used in the treatment of cancer in Kolli Hills south India.

In Nigeria, the decoction of the leaf of *T. rhomboidea* is used traditionally to improve sperm count, treat stomach problems, while the root and bark are used for diarrhea, hemorrhage and gonorrhea; the seed is laxative[5]. In Switzerland, the leaves are taken as vegetable. The plant has also been reported to possess anti-cancer, anti-tumor, anti-oxidant activity, anti HIV activity and anti-viral properties[2,3,6,7]. However, limited information is available on the pharmacological properties of this plant. There is no scientific report on the analgesic and anti-inflammatory effects of this plant to the best of our knowledge, while there are claims on its traditional use for treatment of snake-bite and cancer or tumor. Based on these claims, this study seeks to investigate and establish a scientific report on the analgesic and anti-inflammatory effects of this plant.

MATERIALS AND METHODS

Plant materials

The fresh leaves of *T. rhomboids* were collected in October, 2007, from the local garden within the premises of University of Port Harcourt, Nigeria.

The plant materials were taxonomically identified by Edwin wosu of Botany Herbarium, University of Port Harcourt where Voucher specimen was deposited. The leaves were air-dried until a constant weight was obtained (10 days).

Extraction

The dried leaves were pulverized to fine powder and extracted with methanol in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semi-solid mass obtained concentrated by vacuum drying to yield a solid residue. This was kept in refrigerator for phytochemical and bioassay.

Animals

Male Wister albino rats of weight range 160 – 210 g and male Swiss albino mice of weight range 35 – 45 g were used in this study. The animals were obtained from animal house of University of Port Harcourt. They were grouped and housed in a cage of five animals per cage and allowed to acclimatize with the new environment for 10 days. They were maintained under standard laboratory conditions. The animals were allowed free access to standard dry pellet diet and given water *ad libitum*. All chemicals used are of analar grade.

Phytochemical screening

Chemical tests were carried out on the methanolic extracts and on the powdered specimens using standard procedures to identify the constituents [8,9], by characteristic colour changes as described by Sofowara [10,11].

Anti-inflammatory activity: Egg albumin-induced rat paw oedema

Six groups of rats, each was administered with either plant extract (50, 100, 200 or 400 mg/kg i.p), Piroxicam (0.5 mg/kg i.p) or normal saline as control (5 m/kg) 1 h before the induction of inflammation. Acute inflammation was produced by the subplanter administration of 0.1mL fresh egg albumin into the right hind paw of each rat 1 h after administration of respective extracts. The paw volume was measured at 0 min and 180 mins, taking the readings at 20 mins intervals, after the egg-albumin administration by displacement technique using digital Phlethysmometer [12].

The average volume of the right hind paw of each rat was calculated from four readings which did not deviate more than 3%. The anti-inflammatory effects of the extract was calculated by the following equation:

Anti-inflammatory activity (%) = (1 – D/C) × 100%.

Where D represents the percentage difference in paw volume after extract administration; C represents the percentage difference in volume of the control group[13].

Analgesic activity: Acetic acid induced writhing response in mice

Analgesic effects of the plant extracts were evaluated by Veerappan et al method [14,15] with little modification. Six groups of five mice each were administered by normal saline (5 mL/kg i.p) as control, paracetamol (100 mg/kg i.p) or extract (50,100, 200, 400 mg/kg i.p). 1 h later, 0.6 % acetic acid (10 mL/kg) solution was administered intraperitoneally to all animals in different groups. The number of writhes 5 – 20 mins later after acetic acid injection was counted. A significant reduction of writhes in tested animals compared to control group was considered as an antinociceptic response.

Statistical analysis

Values were expressed as mean ± SEM (n = 5). Statistical analysis was carried out using Graph Pad prism demo software. One way analysis of variance (ANOVA) was used. Values of $P < 0.05$ were considered significant.

RESULTS

In acetic acid-induced writhing test in mice, *T. rhomboidea* caused statistically significant ($P < 0.001$, ANOVA) reduction in the mean number of writhes induced by acetic acid (Table 1, Figure 2). The number of writhes reduced from 64.00 ± 3.40, observed with the group administered by normal saline to 28.17 ± 1.20 in group administered by paracetamol used as standard. *T. rhomboidea* (50 – 400 mg/kg) caused dose-dependent higher reduction in the mean number of writhes than the standard group (Table 1, Figure 1).

In anti-inflammatory activity test, *T. rhomboidea* extract caused statistically significant ($P < 0.001$, ANOVA) inhibition of inflammation induced by egg albumin in the rats paw. The percentage of inhibition of the inflammation caused by the *T. rhomboidea* extract (50 –400 mg/kg) was dose-dependent higher than that obtained with Piroxicam (0.5 mg/kg) which was used as standard (Table 1, Figure 2).

The phytochemical screening indicated the presence of alaloids, flavonoids, terpenoids, saponins, tannins, steroids and volatile oils in the *T. rhomboidea* leaves.

Table 1 The effect of the *T. rhomboidea* extract on acetic acid-induced writhing test in mice and on egg albumin-induced inflammation in rats.

Treatment (mg/kg)	Mean number of writhings	Anti-inflammatory (%)
50	20.20 ± 2.00[a]	59.10 ± 1.70[a]
100	15.00 ± 1.00[b]	68.30 ± 2.60[b]
200	4.70 ± 0.80[b]	82.20 ± 2.60[b]
400	3.00 ± 0.60[b]	91.10 ± 3.60[b]
Normal saline	64.00 ± 3.40	2.80 ± 0.01
Paracetamol 100	28.17 ± 1.20[a]	61.20 ± 1.40[a]

[b]: $P < 0.001$; [a]: $P < 0.05$ significance values (ANOVA); NS normal saline.

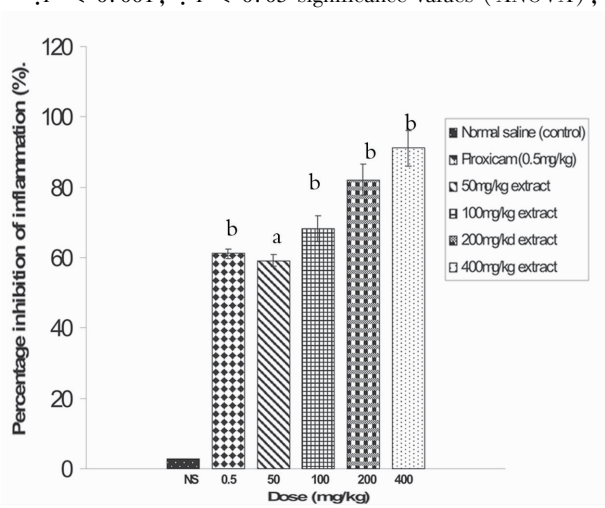

Figure 1 The anti-inflammatory activities of *T. rhomboidea* leaves and Piroxicam (0.5mg/kg) on egg-albumin-induced oedema in the paw of rats.
[b] represents $P < 0.001$ and [a] $P < 0.05$ significance values (ANOVA), NS normal saline.

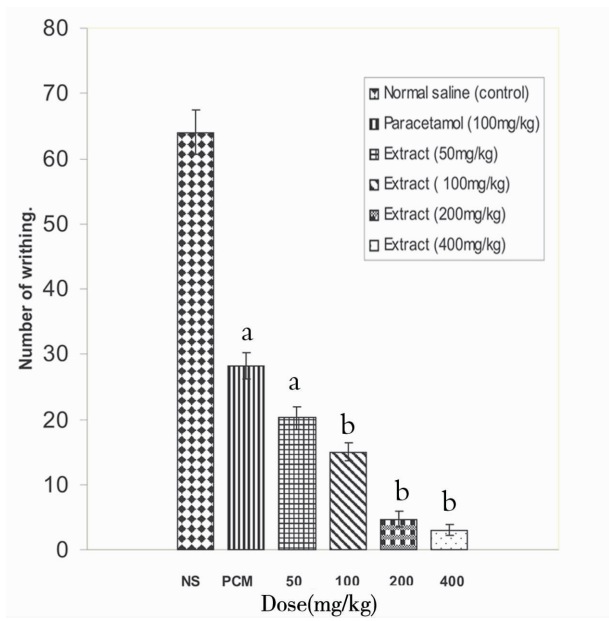

Figure 2 The effect of the methanol extract of *T. rhomboidea* leaves on acetic acid-induced writhing in mice.
[b] represents $P < 0.001$ and [a] $P < 0.05$ significance values (ANOVA)

DISCUSSION

Phytochemical screening of the extract and powdered leaf revealed the presence of flavonoids, saponins,

alkaloids, tannins, volatile oils, steroids and terpenoids.

This study shows that the methanol extract of *T. rhomboidea* possesses a significant anti-oedematogenic effect on egg albumin-induced oedema of the paw of albino rats with $P < 0.001$ (ANOVA). This signifies anti-inflammatory activity. The extract was found to be more active than Piroxicam at higher doses but has the same activity with Piroxicam at lower doses.

Egg albumin-induced inflammation model is a significant predictive test for anti-inflammatory activity[12]. These results are an indication that *T. rhomboidea* can be effective in acute inflammatory disorders.

In acetic acid-induced abdominal writhing which is the visceral pain model [13,15,16], the results show that the extract produced significant analgesic activity at all doses with $P < 0.001$ (ANOVA). The results also show that the extract is more potent than Paracetamol at similar or higher doses. This analgesic effect of *T. rhomboids* can be attributed in part to its anti-inflammatory effect as in visceral pain model the precursor releases arachidonic acid through cycloxygenase and prostaglandin biosynthesis which plays a role in the nociceptive mechanism[16]. Therefore, this implies that the inhibition of acute inflammation by these extracts leads to their inhibitory effect on pain development process[15-17].

The phytochemical analysis of the extract revealed the presence of triterpenoids, steroids, volatile oils, alkaloids, flavonoids, saponnins and tannins. Alkaloids and flavonoids are well known for their ability to inhibit pain perception[15].

Flavonoids as anti-oxidants also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation [18].

Finally, this study confirms the efficacy of *T. rhomboidea* as an analgesic and anti-inflammatory agent, thus gives scientific bases for its traditional uses as anti-snake-bite, anti-cancer and anti-tumor agent. Further study is on the way to isolate, identify and characterize the active constituent responsible for these effects and also to determine the exact mechanism of this action.

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