Evaluation of anti-nociceptive and anti-inflammatory activities of leaf extract of *Turraea vogelli* Hook. f. ex. Benth

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Abstract: The leaf extract of *Turraea vogelii* Hook. f. ex. Benth. is used in ethnomedicine for the management of pain and inflammation. Anti-nociceptive activity was determined using acetic acid-induced mouse writhing model. The anti-inflammatory activity was investigated using *in-vitro* bovine serum albumin (BSA) denaturation assay and BSA-induced hind paw edema in rats. The extract (125-500 mg/kg) administered via the oral route produced a significant (p<0.005) inhibition of acetic acid-induced writhes. The percent inhibition of writhes for extract (500 mg/kg) and diclofenac (10 mg/kg) was 53.3 and 59.5% respectively. The methanol extract (10^{-6}-1.0 µg/mL) inhibited protein denaturation with IC_{50}values of (1.06×10^{-3} µg/mL and 2.58×10^{-3} µg/mL) for extract and diclofenac respectively. Furthermore, the leaf extract (62.5 mg/kg) significantly (p<0.05) inhibited BSA-induced paw edema in rats. The methanol leaf extract of *T. vogelii* has anti-nociceptive and anti-inflammatory activities. These findings justify the use of the plant in traditional medicine for the management of pain and inflammation.

Keywords: Anti-nociceptive, anti-inflammatory, acetic acid, bovine serum albumin, *Turraea vogelli*.

INTRODUCTION

Pain and inflammation are common symptoms of stomach ulcer, infections, cancer, rheumatoid arthritis, heart disease and a host of other diseases (Crowso *et al*., 2013). Inflammation has become the focus of global scientific research because of its implication in virtually all diseases. Steroids, non-steroidal anti-inflammatory drugs (NSAIDs) and opiates are still the mainstay therapy and research is focused on the search for new and safe anti-inflammatory agents (Selvum and Jachak 2004).

Medicinal plants are useful source of bioactive molecules beneficial in the management of pain and inflammation (Kim *et al*., 2004; Shah and Alagawadi, 2011). Majority of people living in developing countries depend on traditional herbal medicine as their primary source of treatment for various illnesses. The affordability of most traditional medicines makes them acceptable at a time of soaring health-care costs and nearly universal austerity (WHO, 2014). Medicinal plants and herbs reported to have anti-nociceptive and anti-inflammatory activity include; *Cassia fistula* (Ilavarsan *et al.*, 2005), *Moringa oleifera* (Sulaiman *et al*., 2008), *Piperovatum* (Vaghasiya *et al*., 2007) and *Bacopa monnieri* (Channa *et al*., 2006).

Turraea vogelii Hook. f.ex. Benth. (*T. vogelii*) is an ethno-medicinal plant indigenous to Tropical Africa. It is a scandent shrub or woody climber up to 5m high widely distributed in evergreen forest and its edges (Burkill, 1985). The leaves, roots and bark are used for urogenital infections, intestinal complaints, and as a decoction for wounds, skin disease, rituals and magic (Qualttroochi, 2012). In Democratic Republic of Congo, the leaves and bark of the plant are powdered and applied for wound healing (Wome, 1985). A recent study reported its anti-proliferative activity on cancer cells (Hamid *et al*., 2015).

There are limited scientific reports on the pharmacological activities of this plant, hence this study was designed to evaluate the anti-nociceptive and anti-inflammatory activities of the methanol leaf extract of *T. vogelli*.

MATERIALS AND METHODS

Collection and identification of plant

The fresh leaves of *T. vogelii* were collected in the month of January 2017 from Onigambari Plantation Reserve, Ibadan, Nigeria. Identification and authentication of the plant was done by Mr. S.A. Odewo of the Forestry Research Institute of Nigeria (FRIN). A voucher specimen with reference number FHI 111265 was deposited at the herbarium of FRIN.

Preparation of plant extract

The leaves were removed from stem and dried under the shade for 2 weeks. The dried leaves were size reduced with a laboratory mill. The powdered leaf (200g) was

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extracted by successive cold maceration with methanol and water in the ratio of 70:30. The resultant mixture was filtered using Whatman filter paper (No.1). The filtrate was evaporated to dryness on a water bath at a temperature of 45°C and the percentage (% w/w) yield was calculated.

Experimental animals

Male Swiss albino mice (20-25 g) and Wistar rats (100-120g) obtained from the animal house of University of Ilorin were used for the study. The animals were allowed to acclimatize for 24 hours with free access to food and water *ad libitium* in the animal house at the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical sciences, University of Ilorin, Ilorin. Ethics clearance was obtained from the University of Ilorin Ethics Review Committee with an approval number UERC/ASN/ 2017/915. All experiments were carried out in accordance with the Guidelines for laboratory Procedures laid down by the University of Ilorin Ethics Committee on Research as well as the International Animal Care and Use Committee (IACUC) in Nigeria.

Preliminary phytochemical screening

Methanol soluble portion of the extract was tested for the presence of; alkaloids, flavonoids, steroids, saponins, tannins and terpenoids (Sofowora, 1993; Trease and Evans 2002).

Determination of anti-nociceptive activity

Anti-nociceptive activity of methanol extract of *T. vogelii* (METV) was evaluated using the method of Fontenele *et al*., 1996 with some modifications. Male Wistar rats were divided into five groups of five rats and treated as follows; Group I: Normal saline (1mL/kg), Group II: diclofenac sodium (10 mg/kg), Groups III, IV and V: 125 mg, 250 mg and 500 mg/kg orally. Acetic acid (1% v/v) was administered intraperitoneally 30 minutes after the treatment to induce pain sensation in the animals. Each mouse was observed inside a glass cupboard. Five minutes after administration of acetic acid, the number of abdominal contractions followed by extension of hind limbs (writhe) was counted for 15 minutes. The numbers of writhes in the treated groups was compared to that of the control group. Percentage inhibition of writhes was calculated thus:

$$\%\text{inhibition} = \frac{(WC-WT)}{WC} \times 100 \text{ where WC} = \text{number of writhes in control group}$$

WT = number of writhes in extract/diclofenac treated groups

Anti-inflammatory activity

Bovine Serum Albumin (BSA) denaturation assay

Anti-inflammatory activity of the leaf extract was evaluated *in vivo* using protein denaturation methods (Williams *et al*., 2008). A test control solution was prepared by adding 50 µL of distilled water to 450 µL of BSA (0.5%). Test solutions contained 50µL of different concentrations of METV (10^{-6}-1µg/ml) and 450µL of BSA (0.5%). The product control was prepared with 50 µL of different concentrations of the extract and 450µL of distilled water. Standard solutions contained 50µL of different concentrations of diclofenac sodium (10^{-6}-1 µg/ml) and 450 µL of BSA (0.5%).

Fig. 1: Leaves, flowers and twigs of *T. vogelii* (flickr.com, 2009)

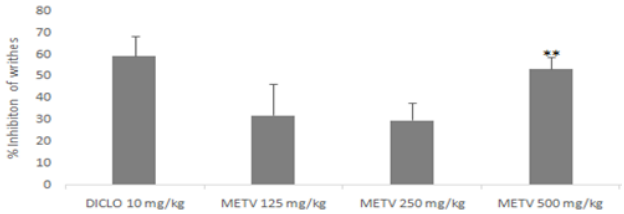

Fig. 2: Inhibitory activity of methanol extract of *T. vogelii* (METV) on acetic acid-induced writhes in mice ***p*<0.005, n=5; Diclo= diclofenac

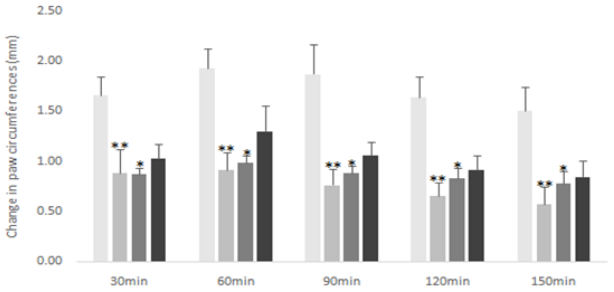

Fig. 3: Inhibitory activity of methanol extract of *T. vogelii* (METV) on bovine serum-induced paw edema *p<0.05, **p<0.005 versus control, n=5 Control, Diclofenac 10mg/kg, METV 62.5 mg/kg, METV 125 mg/kg

The test tubes containing these different solutions were incubated in an oven at 37°C for 20 minutes and at 57°C for another 3 minutes. The samples solutions were allowed to cool and 2.5 mL of phosphate buffer (pH 6.3) was added to all the solutions. The absorbance was measured at 255 nm with GS-UV1PC double beam spectrophotometer. This assay was done in triplicate and the percentage inhibition of protein denaturation was calculated thus:

$$\%\text{ inhibition} = \frac{100-(At-Ac)}{At}$$

Where: At = absorbance of test; Ac = absorbance of control

Table 1: Preliminary qualitative analysis of phytochemicals in methanol extract of *T. vogelii*

Bioactive constituent	Chemical Test	Observation
Alkaloids	Drangendorff's	++
	Wagner's	++
	Meyer's	++
Flavonoids	Shinoda's test	++
	Lead acetate	+
Tannins	$Fecl_3$	++
Saponins	Frothing	+++
	Emulsifying	+++
Anthraquinone	Combined	+++
	Free	+++
Cardiac glycoside	Keller killiani	-
	Kedde	+++
Steroid terpenoid	Salkowski	++

Key (-) absent (+) trace (++) moderate (+++) copious

Table 2: Effect of methanol extract of *T. vogelii* (METV) on protein denaturation

METV µg/mL	% Inhibition	Diclofenac ug/mL	% Inhibition
Control	-	Control	-
10^{-6}	110.80±1.94	10^{-6}	97.23±7.47
10^{-4}	113.88±0.78	10^{-4}	94.39±7.24
10^{-2}	109.69±3.42	10^{-2}	100.66±0.87
10^{0}	112.43±1.88	10^{0}	101.25±2.11

METV= Methanol extract of *T. vogelii*

Bovine serum albumin (BSA) - induced paw edema in rats

Twenty-five male Wistar rats were divided into five groups of five animals and treated as follows: Group I: normal saline (10mL/kg/p.o), Group II: diclofenac sodium (10 mg/kg), Groups III, IV and V: METV 62.5, 125 and 250 mg/kg/p.o. respectively after an overnight fast. Thirty minutes after the treatment, BSA (0.1mL of 0.5% w/v) was administered on the sub-plantar surface of the right hind paw of the rats. Paw circumference for each rat was recorded with a digital Vernier caliper.

STATISTICAL ANALYSIS

Data was expressed as mean ± standard error of mean. The results were analyzed using Graph pad software program version 6.0. Statistical analysis was carried out using One Way Analysis of Variance (ANOVA). Statistical significance was taken at $p<0.05$ and $p<0.005$.

RESULTS

The percentage yield of the hydro methanol leaf extract of *T. vogelii* was 12.75%. Terpenoids, tannins, alkaloids, anthraquinones, cardiac glycosides, flavonoids, steroids and Saponins, terpenoids were present in METV (table 1). Intraperitoneal administration of acetic acid (1% v/v) produced abdominal constrictions in mice. The leaf extract (500 mg/kg) produced a significant ($p<0.005$) reduction in acetic acid-induced abdominal writhes. The percent inhibition in writhes produced by extract (500 mg/kg) and diclofenac sodium (10 mg/kg) was 53.3% and 59.5% respectively (fig. 2). METV produced inhibition of BSA denaturation at concentrations of 10^{-6} – 1 µg/mL. The IC_{50} was calculated to be 1.06 µg/mL × 10^{-3} and 2.58 × 10^{-3} µg/mL respectively for METV and diclofenac respectively (table 2). The anti-inflammatory activity of methanol extract was comparable to that produced by diclofenac sodium. Injection of BSA (0.1 mL of 0.5% w/v) on the sub-plantar surface of the right hind paw of rats produced an increase in paw volume which increased in size and reached a maximum in 60 minutes. METV (62.5 mg/kg) produced a significant ($p<0.05$) inhibitory effect on BSA-induced inflammation (fig. 3). This effect was comparable to that produced by diclofenac (10 mg/kg).

DISCUSSION

In this study the anti-nociceptive and anti-inflammatory activity of methanol leaf extract of *T. vogelii* was investigated for the first time. Flavonoids, tannins and terpenoids present in the extract have been reported to have significant antinociceptive and anti-inflammatory activities (Yanfen *et al*., 2015; Paliwal *et al*., 2017).

Anti-nociceptive activity of METV was evaluated using acetic acid-induced mouse writhing test and the extract produced a significant reduction in acetic acid-induced writhes. Acetic acid induces peripheral pain sensation by release of arachidonic acid, which results in synthesis of cyclooxygenase, prostaglandin and stimulation of peritoneal nociceptors (Neukirch *et al*., 2005, Yu *et al*., 2012). The anti-nociceptive activity of METV maybe mediated through inhibition of release of prostaglandins and other peripheral mediators of pain (Khan *et al*., 2011).

Inhibition of thermally-induced protein denaturation assay was used to assess anti-inflammatory activity of the plant extract. METV at very low concentration produced anti-denaturation effect similar to diclofenac on heat treated bovine serum albumin. Anti-denaturation activity of biologically active compounds and extracts at very low concentrations has been reported (Williams *et al.,* 2008). Thermally-induced bovine serum albumin denaturation results in expression of antigens associated with Type III hypersensitivity reaction which is responsible for inflammatory diseases like rheumatoid arthritis and glomerulonephritis (Rice-Evans *et al*., 1996). At pathological (pH 6.2 – 6.5), non-steroidal anti-inflammatory drugs like diclofenac, indomethacin, and salicylic acid have been reported to prevent denaturation of heat treated BSA (Grant *et al*., 1970 and Paliwal *et al*., 2017).

BSA-induced paw edema method was also used to evaluate anti-inflammatory activity of METV *in vivo*. Acute inflammation induced by BSA was similar to that produced by phlogistic agents such as egg albumin and carrageenan. The mechanism of action of these phlogistic agents has been proposed to be biphasic. The first phase (1-2 hour) is due to liberation of mediators of inflammation like serotonin, histamine and bradykinin, while the second phase is attributed to release of prostanoids and cyclooxygenase (Wang *et al*., 2010; Hassan *et al*., 2015). In the present study, paw edema in rats increased progressively to reach a maximum one hour after injection of BSA. The extract significantly inhibited inflammatory response induced by BSA which suggests an inhibitory effect on release of histamine, serotonin and prostanoids (Masroor *et al*., 2018).

The anti-inflammatory and anti-nociceptive activities of the methanol extract of *T.vogelli* provide a pharmacological justification for the use of the plant extract in management of stomachache, ulcers and wounds.

CONCLUSION

These results show that leaf extract of *T. vogelii* has anti-nociceptive and anti-inflammatory activities and provides support for the ethnomedicinal use of the leaf extract. Further research needs to be carried out to isolate the constituents responsible for anti-nociceptive and anti-inflammatory activities and to elucidate the mechanism of action.

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