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GASTRO-PROTECTIVE ACTIVITY OF *TURRAEA VOGELII* LEAF EXTRACT AGAINST INFLAMMATION INDUCED BY DICLOFENAC

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ABSTRACT

Turraea vogelii Hook f. ex. Benth (Meliaceae) is used in traditional medicine for treatment of gastrointestinal disorders, wounds and infections. The protective effect of methanol leaf extract of *Turraea vogelii* (*T. vogelii*) on gastric inflammation induced by diclofenac was investigated. Rats were treated orally with 200 and 400 mg/kg *T. vogelii* before and after administration of diclofenac (150 mg/kg). Three hours after treatment, animals were anaesthetized with intraperitoneal injection of ketamine (60 mg/kg) and xylazine (7.5 mg/kg). The stomachs were removed for macroscopic examination. The stomach of rats treated with diclofenac showed significant redness, hemorrhagic streak and bands of inflammation. Methanol leaf extract of *T. vogelii* (200-400 mg/kg) significantly (p<0.0001) inhibited gastric inflammation induced by diclofenac. Percentage inhibition of gastric inflammation during pretreatment with *T. vogelii* (200 and 400 mg/kg) was 77.69 % and 83.67 % while inhibition of inflammation with treatment was 86.83 % and 93.66 % respectively. *T. vogelii* leaf extract was found to have potential gastro protective activity on inflammation induced by diclofenac.

KEYWORDS: ***Turraea vogelii*; Leaf extract; Gastro-protective; Acute inflammation; Diclofenac.**

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used in most countries (as prescription and non-prescription medicines) for the management of pain and inflammation [1]. The prevalence of peptic ulcer disease in African countries is high due to frequent use of NSAIDs alone and in locally made herbal preparations [2]. Injury caused to the gastric mucosa by NSAIDs results via inhibition of cyclooxygenase (COX-1 and COX-2), interaction with phospholipids and mitochondrial oxidative phosphorylation. These biochemical processes alter the gastrointestinal barrier resulting in inflammation and ulcers [3].

Traditional medicine is the first-choice health care for majority of populace in developing countries. There has been an increase in its use and this could be attributed to the belief that natural remedies are affordable, accessible and safe [4]. The popular choice of herbal medicines was also reported to be due to treatment successes, a higher tolerability and less side effects [5].

Medicinal herbs containing phytochemicals such as flavonoids, tannins, saponins, and terpenoids have been reported to possess cytoprotective, anti-inflammatory and antioxidant activity [6, 7]. Some of these plants reported to have mucosal protective effect include; *Curcuma longa* [8], *Moringa oleifera* [9], *Zingiber officinale* [10], *Asparagus pubescens* [11].

Turraea vogelii Hook f. ex. Benth (Meliaceae) is a woody climber about 5 meters high. It is commonly called honeysuckle and found in forest margins in West African countries. The leaves are used as spice for cooking and in traditional medicine to treat several ailments including; gastrointestinal

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disorders, urogenital infections and wounds [12]. Reported pharmacological activities of the plant include; antiproliferative [13], anti-inflammatory and anti-nociceptive [14], nutraceutical and topical anti-inflammatory [15]. In addition, acute and sub-acute toxicity studies on methanol leaf extract of *Turraea vogelii* has been reported in literature [16]. There are limited scientific reports on the protective effect of *Turraea vogelii* leaf extract against gastric inflammation induced by diclofenac. The present study thus evaluated the gastroprotective activity of methanol leaf extract of *Turraea vogelii* on diclofenac-induced gastric injury in rats.

MATERIALS AND METHODS

Chemicals

Diclofenac sodium (produced by Acino Pharma AG, Switzerland), omeprazole, normal saline, ketamine, xylazine, methanol (produced by Loba Chemie Pvt, India).

Collection of the plant

The fresh leaves of *Turraea vogelii* were collected in May 2019 from Onigambari Plantation Reserve Ibadan, Oyo State Nigeria. The leaves were identified and authenticated by Mr. SA Odewo at Forestry Research Institute Nigeria (FRIN). Voucher reference number for plant specimen deposited at the herbarium of FRIN was FHI 111265.

Preparation of methanol leaf extract

The leaves were dried under the shade for 2 weeks and reduced to powder with a laboratory mill. A 100 g quantity of the powdered leaves was weighed into a clean jar and macerated in 1.5 L of methanol and water in the ratio of 70:30 for 48 h. This was filtered using a Whatman filter paper (No.1). The filtrate was evaporated to dryness on a water bath at a temperature of 45 ^{o} C.

Animals

Male Wistar albino rats (100-120 g) were obtained from Central Research Laboratory University of Ilorin and allowed to acclimatize in the animal room of Department of Pharmacology and Toxicology. Ethical approval was obtained from University of Ilorin Ethics Review Committee (UERC) with an approval number (UERC/ASN/2019/1860). Laboratory experiments were carried out according to the guidelines of the International Animal Care and Use Committee (IACUC) in Nigeria.

Experimental Protocol

Diclofenac (150 mg/kg) was used to induce gastric inflammation in rats. The method of Trabadela et al.,[17] was employed with modifications.

Pre-treatment with *Turraea vogelii* extract before administration of diclofenac

Thirty male Wistar albino rats were divided into five groups of five animals each. All animals were fasted for 12 hours but allowed free access to water.

Animals were pre-treated as follows; Groups I and II normal saline (10 ml/kg of 0.9 %); group III: omeprazole (20 mg/kg); group IV: *Turraea vogelii* (200 mg/kg), group IV: *Turraea vogelii* (400 mg/kg)**.** One hour after pre-treatment, diclofenac (150 mg/kg) was administered to rats in groups II-V. Three hours after, the rats were anaesthetized with intraperitoneal injection of ketamine (60 mg/kg) and xylazine (7.5 mg/kg). The stomachs were removed for macroscopic examination.

Treatment with *Turraea vogelii* extract after administration of diclofenac

Twenty-five male albino rats were divided into five groups of five animals each. Animals were fasted for 12 hours but allowed free access to water.

Diclofenac (150 mg/kg) was administered to rats in groups II – V and after three hours, the animals were treated as follows; Groups I and II normal saline (10 ml/kg of 0.9 %); group III: omeprazole (20 mg/kg); group IV: *Turraea vogelii* (200 mg/kg), group IV: *Turraea vogelii* (400 mg/kg)**.** Three hours after treatment, animals were anaesthetized with intraperitoneal injection of ketamine (60 mg/kg) and xylazine (7.5 mg/kg). The stomachs were removed for macroscopic examination.

Macroscopic evaluation of isolated stomach

The stomachs of the rats were opened along the greater curvature to gain access to the inner lining of gastric mucosa. The gastric content was rinsed with normal saline (0.9 %). The washed stomachs were placed on a white plain sheet of paper and gastric mucosa examined with a 10×4 magnifying lens [18]. Damage to the gastric mucosa appeared as red coloration, haemorrhagic streaks and bands of inflammation.

Scoring of inflammation

Mucosa inflammation was determined using a 0 – 3 scoring method [19] and recorded as;

Normal colored stomach (0)
Red coloration (1)
Hemorrhagic streak (2)
Bands of Inflammation (3)

$$Inflammation\ index\ (II) = \frac{Total\ inflammation\ score}{No.\ of\ animals\ with\ inflammed\ mucosa}$$

$$\%\ inhibition\ of\ inflammation = \frac{II\ control - II\ test}{II\ control}\ x\ 100$$

Statistical analysis

Data were expressed as mean ± S.E.M. and analyzed using Graph Pad Prism (Version 8). Statistical analysis comparing the control and treatment groups was carried out using one-way ANOVA. Statistical significance was taken at $p < 0.0001$.

RESULTS

Pre-treatment with *Turraea vogelii* extract on diclofenac-induced inflammation

Macroscopic examination of gastric mucosa in control group I, showed no redness, hemorrhagic streak or bands of inflammation (Figure 1A). The mucosa of animals in group II, administered diclofenac alone had significant degree of redness, hemorrhagic streaks and bands of inflammation (Figure 1B). There was significant ($p < 0.0001$) inhibition in redness, hemorrhagic streaks and bands of inflammation in groups IV and V animals pre-treated with 200 and 400 mg of *T. vogelii* extract.

Administration of diclofenac (150 mg/kg) to rats in group II caused inflammation of gastric mucosa. Pre-treatment of rats in groups IV and V with extract of *T. vogelii* (200 and 400 mg/kg) significantly ($p < 0.0001$) inhibited gastric inflammation by 77.69% and 83.67% respectively. Omeprazole (20 mg/kg) produced an inhibition of 75.30% (Table1).

Treatment with *Turraea vogelii* extract on diclofenac-induced inflammation

Macroscopic examination of stomach of rats in group I (control) showed no redness, hemorrhagic streak or bands of inflammation (Figure 2A). Rats in group II administered diclofenac, had stomach with significant redness, hemorrhagic streaks and bands of inflammation (Figure 2B). There was a reduction in inflammation in group III treated with omeprazole (Figure 2C). In addition, the stomach of rats in groups IV and V administered diclofenac and treated with (200 and 400 mg/kg) *T. vogelii* showed significant reduction in redness, hemorrhagic streaks and bands of inflammation (Figures 2D and E).

Treatment of rats with leaf extract of *T. vogelii* (200 and 400 mg/kg) after administration of diclofenac resulted in significant ($p< 0.0001$) dose-related reduction in inflammation. The percentage inhibition of inflammation after treatment with the extract was 86.83 % and 93.66 % respectively. Omeprazole (20 mg/kg) produced a percentage inhibition of 74.15 % (Table 2).

DISCUSSION

Alternative therapies from herbal sources are explored globally for the management of several ailments. Increased popularity and use of herbal remedies has resulted in research efforts to ascertain the efficacy of herbal medicines [20, 21]. The leaf extract of *Turraea vogelii* is used in Traditional medicine for treatment of gastrointestinal disorders including gastritis and dyspepsia [12].

In the present study, the protective effect of the leaf extract of *Turraea vogelii* against gastric inflammation induced by diclofenac was evaluated. NSAIDs have been reported to produce topical irritation on the epithelium and bleeding which plays an important role in the development of gastric inflammation [22].

Oral administration of diclofenac caused damage to gastric mucosa in rats characterized by redness, hemorrhagic streaks and bands of inflammation.

Treatment with methanol leaf extract of *Turraea vogelii* produced significant inhibition of gastric inflammation induced by diclofenac. The percentage inhibition of inflammation by the extract was comparable to standard drug omeprazole. Macroscopic features of gastric mucosa of rats treated with methanol leaf extract of *T. vogelii* (200 and 400 mg/kg) showed no redness, hemorrhagic streaks and bands of inflammation compared to the group administered diclofenac. Results obtained from this study revealed the methanol leaf extract of *T. vogelii* has protective activity on gastric inflammation induced by diclofenac. The mucosa protective effect of the extract was observed to be dose-dependent in both pre- and treatment phases of experiment.

Diclofenac inhibits the synthesis of prostaglandins by inhibiting COX-1 and COX-2 and plays a significant role in the development of gastric inflammation because of its ability to disrupt the mucosal defensive factors. Prostaglandins play an important role in protecting the gastrointestinal mucosa against harmful effects of toxic agents and NSAIDs [23]. They produce gastroprotective activity by inhibiting the release of inflammatory mediators such as histamine from mast cells [19]. The mucosa protective effect of methanol leaf extract of *T. vogelii* may be due to its ability to reduce topical irritation on the epithelium [22] and inhibit release of inflammatory mediators [24].

Plants containing phytochemicals such as flavonoids have been reported to have anti-inflammatory [4] and antiulcer activity [25]. A previous study on the phytochemical constituents of *T. vogelii* leaf extract revealed the presence of phenolic compounds and

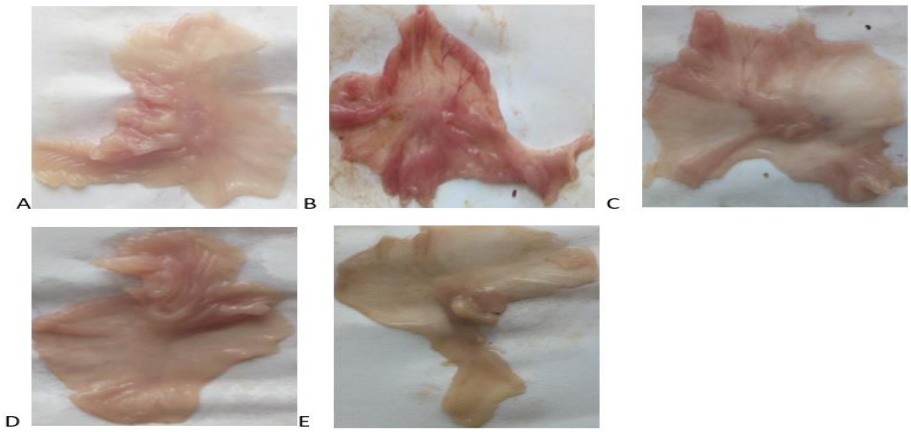

Figure 1: Macroscopic findings in stomach of rats pre-treated with extract. 40×magnification.
A: Control; B: Diclofenac 150 mg/kg; C: Omeprazole 20 mg/kg; D: *T. vogelii* 200 mg/kg; E: *T. vogelii* 400 mg/kg.

Table 1: Effect of pre-treatment with *Turraea vogelii* extract on diclofenac-induced inflammation

Treatment	Dose	Mean Inflammation Index	% Inhibition
Normal Saline	1 ml/kg	0.00 ± 0.00	-
Diclofenac	150 mg/kg	50.20 ± 5.00	-
Omeprazole	20 mg/kg	12.40 ± 3.44*	75.30
T. vogelii extract	200 mg/kg	11.20 ± 2.42*	77.69
T. vogelii extract	400 mg/kg	8.20 ± 2.91*	83.67

Data are mean ± SEM, *$p < 0.0001$, n=5

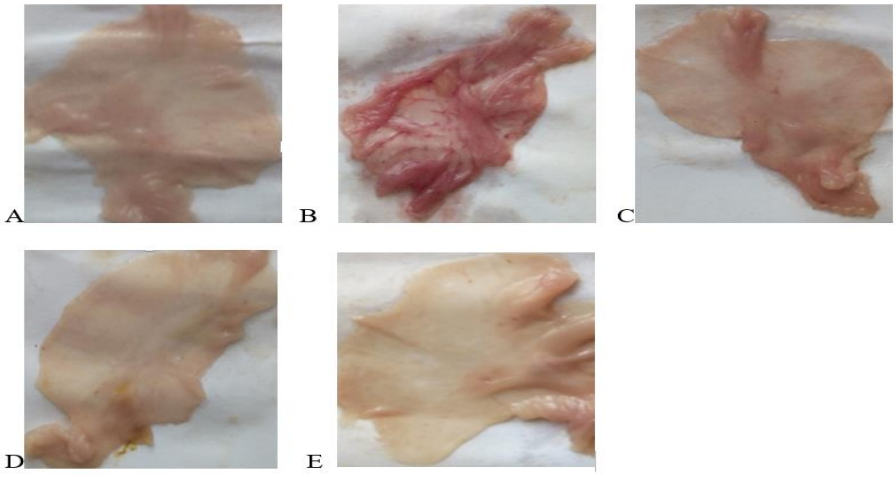

Figure 2: Macroscopic representation of stomachs in rats treated with extract. 40× magnification.
A: Control, B: Diclofenac 150 mg/kg, C: Omeprazole 20 mg/kg, D: *T. vogelii* 200 mg/kg, E: *T. vogelii* 400 mg/kg.

Table 2: Effect of treatment with *Turraea vogelii* extract

Treatment	Dose	Mean Inflammation Index	% Inhibition of inflammation
Normal Saline	1 ml/kg	0.00 ± 0.00	-
Diclofenac	150 mg/kg	41 ± 5.73	-
Omeprazole	20 mg/kg	10.60 ± 2.49*	74.15
T. vogelii	200 mg/kg	5.40 ± 1.44*	86.83
T. vogelii	400 mg/kg	2.60 ± 1.17*	93.66

Data are mean ± SEM, *p<0.0001, n=5

flavonoids which may be responsible for its anti-inflammatory activity [15, 26]. The mucosal protective effect of *T. vogelii* may be attributed to phytochemical constituents present in the leaf extract.

CONCLUSION

Results from the study show that the methanol leaf extract of *Turraea vogelii* inhibited gastric inflammation and redness induced by diclofenac and thus has a mucosa-protective activity. Further studies will be carried out to isolate the potential active compounds and determine the mechanism of action.

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