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Short Communication

Elucidation of the Possible Mechanism of Antinociceptive Action of Methanol Stem Bark Extract of *Zanthoxylum gilletii* (De Wild.) in Mice

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ABSTRACT

Background and Purpose: *Zanthoxylum gilletii* (De Wild.) stem bark is used traditionally for pain management, but its antinociceptive activity is yet to be validated. This study evaluated the antinociceptive activity and elucidated the possible mechanism of antinociceptive action of its methanol stem bark extract.

Methods: Oral acute toxicity was evaluated using Lorke's method, while antinociceptive activity was assessed using acetic acid-induced writhing test. Mice were randomly assigned to groups receiving distilled water, morphine (10 mg/kg), or the extract (250 and 500 mg/kg). Mechanistic studies involved pre-treating mice with naloxone (2 mg/kg), glibenclamide (5 mg/kg), or propranolol (20 mg/kg) 15 min before the extract (500 mg/kg), followed by acetic acid administration.

Results: No mortality or signs of toxicity were observed at acute oral doses up to 5,000 mg/kg. The extract significantly (P<0.01) reduced writhing counts in a dose-dependent manner, with effects reversed by naloxone (P<0.05), enhanced by propranolol and unaffected by glibenclamide.

Conclusion: *Zanthoxylum gilletii* extract has a high oral safety margin, with an LD_{50} exceeding 5,000 mg/kg. The antinociceptive activity of the extract may involve opioid receptor activation, but not beta-adrenergic pathway or ATP-sensitive potassium channels. Further studies would determine its bioactive components.

Pain management remains a critical area for therapeutic intervention, with opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) being the two major classes of analgesics often used, despite their associated adverse effects such as gastric ulceration, tolerance, and dependency (Alorfi, 2023). Natural products derived from medicinal plants are often a rich source of novel analgesics with fewer adverse effects (Arena *et al*., 2022). One widely used analgesic medicinal plant is *Zanthoxylum gilletii* (De Wild.)**.**

Z. gilletii is commonly used in traditional African medicine for the treatment of pain, inflammation, infections, and digestive disorders (Okagu *et al*., 2021). Its stem bark, in particular, is believed to have potent therapeutic effects and is often utilized in decoctions or topical applications for pain relief, which suggests a possible analgesic action (Mbula *et al*., 2022). The stem bark extract has been used to treat toothache, rheumatism, and digestive issues (Siddiqui, 2011).

The genus *Zanthoxylum* is known for its rich chemical composition, including tannins, alkaloids, essential oils and flavonoids. These compounds are associated with various pharmacological effects, such as analgesic and anti-inflammatory actions (Okagu *et al*., 2021; Mbula *et al*., 2022).

In view of the above, this present study was to evaluate the antinociceptive activity of *Z. gilletii* stem bark extract and investigate the possible mechanism(s).

MATERIALS AND METHODS

Plant Collection, Identification and Extraction

The stem bark of *Z. gilletii* was collected from *Kizinda* village-Ishaka municipality, Bushenyi district, Western Uganda. The plant was identified and authenticated Dr. Olet Eunice of the Department of Botany, Mbarara University of Science and Technology, Uganda, and its identity was confirmed by comparison with a previously deposited specimen (SM20) in the Department of Biology, Faculty of Science, Mbarara University of Science and Technology, Uganda. The bark was air-dried, pulverized, and 1 kg of the powdered material was macerated in 5 L of methanol for 72 h at room temperature with intermittent shaking. The resulting extract was filtered and concentrated under reduced pressure using a rotary evaporator (RE-52CS ZX: Shanghai Zhixun) to obtain a dark brown, sticky residue. The percentage yield of the extract was calculated.

Experimental Animals

Healthy adult mice (20–30 g) of either sex were obtained from the animal facility of the Department of Pharmacology, Kampala International University, Uganda. The animals were housed under standard laboratory conditions with free access to water and commercial feed. To minimize stress and ensure physiological stability, they were allowed to acclimatize for two weeks prior to the commencement of the experiment. Throughout the study, the handling and treatment of mice adhered strictly to internationally recognized and established guidelines for the humane use of laboratory animals (Office of Laboratory Animal Welfare, 2002). The approval for the research was granted by the Research and Ethics Committee of Kampala International University (KIU-2019-06). In addition, the National Institutes of Health's Guide for the Care and Use of Laboratory Animals criteria was followed (Publication No. 80-23, revised 1996).

Drugs and Chemicals

Glacial acetic acid (Sigma-Aldrich, USA), propranolol hydrochloride (Sigma-Aldrich, USA), glibenclamide (Sigma-Aldrich, USA), naloxone hydrochloride (Abcam Biochemicals Plc, Cambridge, UK), morphine sulphate (MacFarlan Smith, UK), and absolute methanol (Fisher Scientific, UK) were of analytical grade. Except for methanol, which was used for extraction, all test agents were freshly prepared in distilled water (or 0.5% carboxymethyl cellulose, where applicable) and administered orally at appropriate doses in a volume of 10 mL/kg body weight.

Acute Toxicity Studies

The oral acute toxicity of *Z. gilletii* was assessed using the Lorke (1983) method. In the initial phase, mice (n=3) were administered graded doses (10, 100, and 1,000 mg/kg) of *Z. gilletii* extract, while in the phase-2, mice (n=1) were treated with higher doses (1,600, 2,900, and 5,000 mg/kg) of the extract. The mice were closely monitored for physical signs of toxicity, including alterations in behavior, neurological symptoms, gastrointestinal disturbances, as well as mortality, over a 24-h period with closer observation during the first 4 h. The median lethal dose (LD_{50}) value was subsequently determined using the formula below:

$$LD_{50} = \sqrt{(\text{Highest dose with no deaths} \times \text{Lowest dose with deaths})}$$

Evaluation of the Antinociceptive Activities and Possible Mechanism of Antinociceptive Action of the Extract

The acetic-acid-induced writhes test was used to evaluate the antinociceptive activity (Koster *et al*., 1959). Four groups of mice (n=5) orally received distilled water (10 mL/kg), morphine (10 mg/kg), or the extract (250 and 500 mg/kg). Mechanistic studies involved pre-treating mice with propranolol (20 mg/kg), glibenclamide (5 mg/kg), or naloxone (2 mg/kg), 15 min before the extract (500 mg/kg) (Odoma *et al*., 2017). One hour after administering the substances, each mouse received an intraperitoneal

injection of 0.6% v/v acetic acid (10 mL/kg). The mice were observed for 10 min, starting 5 min after the acetic acid injection, and the number of abdominal writhes was recorded. A decrease in the number of writhes in contrast to the distilled water treated group was taken as an indication of antinociceptive activity. The percentage inhibition of writhes was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Mean number of writhes (Distilled water)} - \text{Mean number of writhes (Test)}}{\text{Mean number of writhes (Distilled water)}} \times 100$$

Statistical Analysis

The results are presented as mean values ± standard error of the mean (SEM). GraphPad Prism software (version 8) was used for one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. A *P*-value of less than 0.05 was considered a statistically significant difference between the compared data.

RESULTS

Acute Toxicity Studies

The oral acute toxicity test showed that the median lethal dose of *Z. gilletii* methanol extract in mice was higher than 5000 mg/kg. Notably, even at increasingly high doses, the mice did not exhibit any visible signs of toxicity, such as altered mental state, gastrointestinal disturbances, seizures, or tremors.

Antinociceptive Property

Writhing was observed in mice following acetic acid injection, with an average of 44.4±1.29 writhes recorded over a 10-min period, in the distilled water treated group. The extract and morphine treated groups had a significant reduction in the number of writhes (P<0.01; P<0.001) compared with the distilled water group. Maximum inhibition were 54.05%, 71.62%, and 93.20% for 250 mg/kg (extract), 500 mg/kg (extract), and 10 mg/kg (morphine) respectively (Figure 1).

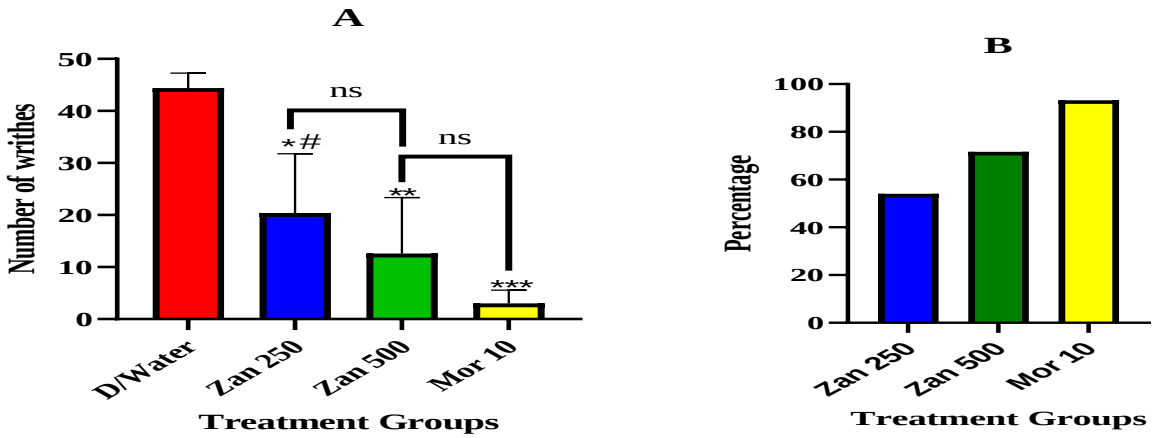

Figure 1: Effects of methanol stem bark extract of *Z. gilletii* on acetic acid induced writhing in mice (A), and percentage inhibition of pain (B). *P<0.01; **P< 0.001, ***P<0.0001, $^{\#}P$< 0.01versus morphine group D/Water = Distilled water, Zan = *Zanthoxylum gilletii* extract, Mor = Morphine. Figures after each abbreviation represent doses in mg/kg. n=5.

Mechanistic Investigation

While pretreatment of the mice with naloxone significantly (P<0.05) increased the number of writhes, pretreatment with propranolol significantly decreased it (Figure 2). Glibenclamide had no effect on the number of writhes when it was used for pretreatment.

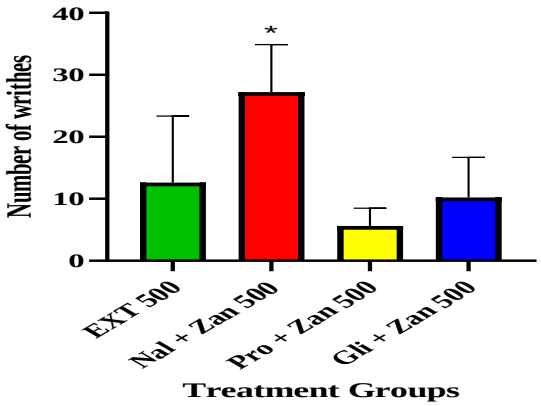

Figure 2: Mechanism of antinociceptive effect of methanol stem bark extract of *Zanthoxylum gilletii*. $^{*}P$<0.05 versus Zanthoxylum (EXT 500) group DW= Distilled water, Nal = Naloxone, Pro = Propranolol, Gli = Glibenclamide. Figures after each abbreviation are doses in mg/kg. n=5.

DISCUSSION

This study sought to evaluate the pain-relieving properties of the methanol stem bark extract of *Zanthoxylum gilletii*. A phytochemical analysis revealed a diverse array of bioactive compounds, including alkaloids, tannins, flavonoids, glycosides, carbohydrates, and saponins many of which have been previously implicated in the antinociceptive activities of various medicinal plants (Kore *et al*., 2010; Mbula *et al*., 2022).

Acute toxicity testing such as the median lethal dose (LD_{50}) determination, is a preliminary step in evaluating the safety profile of medicinal plant extracts. The LD_{50} provides a quantitative estimate of acute toxicity and helps to identify safe dosage ranges for pharmacological studies (Lorke, 1983). Oral treatment with *Z. gilletii* at doses up to 5000 mg/kg in mice did not induce mortality or visible signs of toxicity, suggesting a favorable safety profile for oral administration (Van-Noordwijk and Van-Noordwijk, 1988).

The acetic acid-induced writhing test is a well-established model for evaluating peripheral antinociceptive activity in rodents. This assay induces abdominal constrictions (writhes) via the release of endogenous mediators such as prostaglandins and bradykinin, which activate peripheral nociceptors (Koster *et al*. 1959; Le Bars *et al.,* 2001). The extract exhibited a dose-dependent decrease in acetic acid-induced abdominal writhes, indicating a peripherally mediated antinociceptive effect. The antinociceptive effect of *Z. gilletii* stem bark extract seems to involve the opioid naloxone-sensitive pathway, as evidenced by the reduction of its antinociceptive effect by naloxone, a non-selective opioid receptor antagonist (Amrani *et al*., 2011). Opioid receptors, situated in the peripheral and central nervous systems, play a crucial role in pain modulation by binding with endogenous opioid peptides, that are involved in various physiological processes (Ferrari *et al*., 2012).

In contrast, the antinociceptive activity of *Z. gilletii* stem bark extract does not appear to involve the beta-adrenergic or K^+-ATP channel pathways, as pretreatment with propranolol, a non-selective beta-blocker, and glibenclamide, an ATP-sensitive potassium channel blocker, did not significantly affect its antinociceptive activity. Ion channels play a crucial role in pain signal generation and neuronal excitability, and K^+ channel opening can exert antinociceptive effects by hyperpolarizing neuronal membranes, thereby reducing excitability and inhibiting neurotransmitter release (Palkovits, 2000; Zakaria and Vassar, 2018). Since beta-adrenoceptor activation is often associated with pronociceptive responses—facilitating pain transmission—the lack of effect of propranolol suggests that the extract's antinociceptive action is likely independent of beta-adrenergic arm (Millan, 2002; Petit *et al*., 2013).

CONCLUSION

Conclusively, this study provides evidence for the folkloric antinociceptive property of the methanol stem bark extract of *Zanthoxylum gilletii*, which seems to be mediated through the activation of the opioid naloxone-sensitive pathway. *Z. gilletii* is a potential source of novel analgesic agents. Additional studies would further elucidate the mechanisms of action and explore the therapeutic potential of *Z. gilletii* of stem bark extract.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTION

SO conceptualization, designed and supervised the study. GB carried out experiments, data analysis, and writing. Both authors read the manuscripts and approved the final version for publication.

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AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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