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# ACUTE TOXICITY TEST AND EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF METHANOL LEAF EXTRACT OF *OCHNA KIBBIENSIS* HUTCH AND DALZ (OCHNACEAE)

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## ABSTRACT

Pain is an unpleasant feeling caused by the stimulation of pain receptors. Inflammation is a complex response to exogenous and endogenous stimuli. Ochna kibbiensis Hutz and Dalz (Ochnaceae) is majorly used traditionally in the treatment of pain, inflammation and malaria. This study aimed to evaluate the analgesic and anti-inflammatory properties of the leaf extract of the plant. The analgesic activity of the Methanol leaf extract (MLE) was evaluated using acetic acid-induced writhing in mice and formalin-induced pain in rat models while the anti-inflammatory studies were carried out using formalin-induced inflammation in rats. In the acetic acid model, the MLE in graded doses (40, 80 and 160 mg/kg) significantly ($p<0.05$) and in a concentration-dependent manner reduced the number of writhes induced by acetic acid. Similarly, the extract significantly ($p<0.05$) reduced pain induced by formalin in both phases, with the highest dose (160 mg/kg) having a higher % inhibition than the standard drug, Piroxicam (10 mg/kg). In the formalin-induced inflammation test in rats, at the fourth hour, all the doses significantly ($p<0.05$) in a concentration-dependent manner reduced the paw oedema of the rat. To the best of our knowledge, this is the first report of the evaluation of the analgesic and anti-inflammatory activities of the methanol leaf extract of O. kibbiensis.

**Keywords**: *Ochna kibbiensis*, Toxicity, Antinociceptive, Analgesic, Anti-inflammatory

## INTRODUCTION

Pain is the most common reason for people to use complementary and alternative medicine as well as physician consultation in most developed countries (Debono *et al.,* 2013). Inflammation is the complex response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants (Oronsky *et al*., 2022). Many inflammatory diseases are major cause of morbidity and mortality (Orlando and Mainous, 2024). Pain and Inflammation have been indicated in several diseases including cancer (Zhang *et al.,* 2024); they are part of the major symptoms in many medical conditions, and can interfere with a person's quality of life and general functioning (Zheng *et al.,* 2023), with chronic pain affecting between 30 and 50 % of the world population (Barcellos, 2017).

The non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics are the commonly used drugs of choice for the treatment of pain and inflammatory diseases (Magadum *et al.,* 2015; Bature *et al.,* 2022), but the drugs are accompanied by many side effects such as gastro intestinal disturbances, ulceration, impairment of renal functions, hypersensitivity reactions, addiction, tolerance, dependence, respiratory depression, renal damage, amongst others (Magadum *et al.,* 2015). The above drawbacks necessitate the search for newer agents that are accessible with fewer side effects.

Different medicinal plants have been screened for their effect on pain and inflammation (Bushra and Ganga, 2003; Abdullahi *et al*., 2024). Some species of *Ochna* were reported to have analgesic, anti-inflammatory, anticancer, anti-HIV and anti-atherogenic activities (Suh *et al*., 2006; Kumar and Srinivas, 2022). *Ochna kibbiensis* Hutz and Dalz (Family; *Ochnaceae*) is a shrub or small tree found in tropical Africa from Guinea to southern and northern Nigeria, it is characterized by the presence of elliptical, lanceolate leaves which acuminate at the apex with paired and axillary flowers as well as large, brilliant red calyx in the fruit (Abdullahi *et al*., 2016). The plant is reportedly used in ethnomedicine as an anticancer, anti-malarial, laxative, antiseptic, stimulant, febrifuge, among others (Burkill, 1985). Pharmacologically, it has been reported to possess antimicrobial (Abdullahi *et al*., 2015) and anti-proliferative (Abdullahi *et al*., 2016) activities. Preliminary Phytochemical screening conducted on the methanol leaf extract of *O. kibbiensis* revealed the presence of steroids, triterpenes, flavonoids, alkaloids, saponins, tannins and glycosides (Abdullahi *et al.,* 2015). Ochnaflavone was isolated from the leaf of the plant (Abdullahi *et al*., 2016).

## MATERIALS AND METHODS

### Plant sample collection

The plant material (*Ochna kibbiensis*) was collected from Samaru, Sabon Gari Local Government Area of Kaduna state, Nigeria in October 2017 and was authenticated by Namadi Sanusi of the Herbarium Section, Department of Botany, Ahmadu Bello University, Zaria by comparing with a specimen with voucher number (573). The leaves were removed, shade dried, pulverized and stored at 35 °C for further use.

### Extraction procedure

Pulverized plant material (525 g) of *O. kibbiensis* leaves was macerated in 3 litres of methanol for three days with occasional shaking/swirling. The extract was decanted, filtered and concentrated using rotary evaporator at 40 °C to afford a dark-greenish methanol leaf extract (MLE).

### Experimental Animals

Locally bred adult Swiss albino mice (18-28 g) and adult Wister rats of either sex weighing 121-222 g were obtained from the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Bayero University Kano, Nigeria.

They were fed with laboratory diet and water *ad libitum* and maintained under standard conditions (35 ºC) in propylene cages at room temperature.

**Acute toxicity studies ($LD_{50}$) in mice**

The method of Lorke (1983) was adopted for this study. It was divided into two phases; in the first phase nine mice were divided into three groups (1, 2 and 3) containing three mice each and were injected (*i.p*) with 10, 100 and 1000 mg/kg of crude methanol leaf extract (MLE), respectively. In the second phase, four mice were divided into four groups and were given 200, 400, 800 and 1600 mg/kg (*i.p*) and observed for signs and symptoms of toxicity and mortality for 24 h. The $LD_{50}$ was calculated using the formula; $LD_{50} = \sqrt{\text{minimum lethal dose} \times \text{maximum tolerated dose}}$

**Analgesic studies**

***Acetic acid induced writhing in mice***

The test was conducted according to the method described by Koster *et al.* (1959). Twenty-five (25) mice were divided into five (5) groups of 5 mice each. Group 1 (negative control) was injected *i.*p with 10 mL/kg of normal saline; groups 2, 3 and 4 were treated *i.p*. with 40, 80 and 160 mg/kg of MLE respectively, while group 5 (positive control) received 10 mg/kg piroxicam. Thirty minutes later, each mouse received 10 mL/kg of acetic acid (0.6 % v/v) solution *i.p*. After 5 minutes, the mice were observed for a number of abdominal constrictions for a period of 10 minutes. Percentage inhibition of writhing was determined using the formula;

$$\% \text{ mean inhibition} = \frac{\text{Mean of writhing (control)} - \text{Mean of writhing in (test group)}}{\text{Mean of writhing (control)}} \times 100$$

***Formalin-induced pain in rats***

The test was conducted according to the method described by Dubuisson and Dennis (1977) and modified by TjØlsen *et al*. (1992). Twenty-five (25) adult Wister rats were divided into 5 groups of 5 rats each. Group 1 was injected with 10 mL/kg of normal saline (negative control); groups 2, 3 and 4 were injected with 40, 80 and 160 mg/kg of the MLE respectively, while group 5 received 10 mg/kg of piroxicam *i.p* which served as the positive control. Thirty minutes later, each rat was treated with formalin (50 µl of 2.5 % v/v solution) under the plantar surface of the right hind paw. Licking time in seconds was then recorded after the first 5 min and then after 45 min from the time of the injection of formalin.

**Anti-inflammatory studies**

***Formalin induced-inflammation in rats***

Anti-inflammatory study was carried out according to the method described by Sayya *et al.* (2003). Groups 1, 2, 3, 4 and 5 were injected *i. p.* with 10 mL/kg of normal saline, 40, 80 and 160 mg/kg of MLE and 10 mg/kg of piroxicam, respectively. Thirty minutes later, each mouse was injected with (50 µL of 2.5 % v/v solution) in the sub plantar region of the left hind paw. The paw diameter (cm) was measured using vernier caliper before injection (0 h) and after injection at interval of 60 minutes for four hours.

**Statistical Analysis**

The results obtained were expressed as mean ± SEM. The mean values of the control group were compared with the mean values of treated groups using one-way ANOVA, followed by post hoc (Dunnett t-test); and values were considered significant at $p<0.05$

**RESULTS AND DISCUSSION**

**Acute toxicity studies of methanol leaf extract (MLE)**

The intraperitoneal median lethal dose ($LD_{50}$) of methanol leaf extract was found to be 565.69 mg/kg (Table 1).

**Table 1: Median Lethal Dose of Methanol Leaf Extract (MLE)**

| Doses (mg/kg) | Number of mice used | Mortality |
|---|---|---|
| **First phase** | | |
| 10 | 3 | 0/3 |
| 100 | 3 | 0/3 |
| 1000 | 3 | 2/3 |
| **Second phase** | | |
| 200 | 1 | 0/1 |
| 400 | 1 | 0/1 |
| 800 | 1 | 1/1 |
| 1600 | 1 | 1/1 |

**Analgesic studies**

***Acetic acid-induced writhing test***

The methanol leaves extract significantly ($p<0.05$) and dose-dependently reduced the number of writhes induced by acetic acid by 53, 58 and 71 % at 40, 80 and 160 mg/kg respectively. The highest dose (160 mg/kg) exhibited maximum inhibition of writhes (71 %) which was higher than the standard drug, piroxicam with 62 % (Table 2).

**Table 2: Effect of MLE on Acetic Acid-Induced Writhing Test in Mice**

| Treatment (mg/kg) | Mean ± SEM | % Inhibition |
|---|---|---|
| N/saline | 31.60±0.87 | - |
| MLE (40) | 14.80±0.66* | 53 |
| MLE (80) | 13.20±1.11* | 58 |
| MLE (160) | 9.20±0.37* | 71 |
| Piroxicam (10) | 12.00±1.66* | 62 |

MLE = methanol leaf extract, N/saline = normal saline, SEM = standard error mean

Each value represents mean ± SEM.

* The mean difference compared with negative control is considered significant at $p<0.05$ level

***Formalin-induced pain in rats***

There was a significant ($p<0.05$) reduction of pain induced by formalin after administration of the methanol leaf extract of *O. kibbiensis* at the graded doses (40, 80 and 160 mg/kg). The extract at 160 mg/kg significantly reduced pain in rats with 79 and 88 % inhibition in the first and second phases respectively which was higher than the standard drug, piroxicam (10 mg/kg) having 72 and 85 % inhibition (Table 3).

**Table 3: Formalin Induced-Pain in Rats**

| Treatment | Dose (mg/kg) | First phase | | Second phase | |
|---|---|---|---|---|---|
| | | Mean of writhing | % inhibition | Mean of writhing | % inhibition |
| N/S | 1ml/kg | 10.60±1.80 | - | 13.40±3.26 | - |
| MLE | 40 | 3.60±0.60* | 66 | 3.00±0.00* | 78 |
| MLE | 80 | 3.00±0.44* | 72 | 2.40±0.24* | 82 |
| MLE | 160 | 2.20±0.37* | 79 | 1.60±0.24* | 88 |
| Piroxicam | 10 | 3.00±0.44* | 72 | 2.00±0.31* | 85 |

MLE = methanol leaf extract, N/S = normal saline, SEM = standard error mean
The mean difference is significant at $p<0.05$ level.

**Anti-inflammatory studies**

***Formalin-induced inflammation in rats***

The plant *O. kibbiensis* has demonstrated a significant ($p<0.05$) anti-inflammatory effect. The extract (160 mg/kg) exhibited 100 % inhibition of inflammation induced by formalin in rats at the 4th hour compared to the normal saline control (Table 4).

**Table 4: Percentage Inhibition of Formalin Induced-Paw Edema in Rats**

| Treatment | Dose (mg/kg) | Mean Paw Diameter (cm) ± SEM (% Inhibition) | | | |
|---|---|---|---|---|---|
| | | 1 h | 2 h | 3 h | 4 h |
| N/S | 10 | 0.16±0.02 | 0.18±0.01 | 0.17±0.02 | 0.17±0.02 |
| MLE | 40 | 0.15±0.02 (6) | 0.17±0.01 (6) | 0.12±0.01 (29) | 0.07±0.00* (59) |
| MLE | 80 | 0.09±0.01 (44) | 0.10±0.02 (44) | 0.08±0.02* (53) | 0.04±0.00* (76) |
| MLE | 160 | 0.03±0.01* (81) | 0.04±0.01* (78) | 0.02±0.00* (88) | 0.00±0.00* (100) |
| Piroxicam | 10 | 0.06±0.01* (63) | 0.08±0.01* (56) | 0.03±0.00* (82) | 0.01±0.00* (94) |

MLE = methanol leaf extract, n/saline = normal saline, SEM = standard error mean
The mean difference is considered significant at $p<0.05$ level.

**Discussion**

The $LD_{50}$ of methanol leaf extract of *O. kibbiensis* was found to be moderately toxic (565.69 mg/kg) when administered intraperitoneally. A higher $LD_{50}$ value was reported for *O. scweinfurthiana* (Ibrahim *et al.,* 2015). Thus, the toxic nature of the plant might be attributed to the nature of constituents detected in the plant (Abdullahi *et al.,* 2015).

The acetic acid writhing model is very sensitive, compared to other methods, in detecting the anti-nociceptive effect of compounds (Shumaia *et al*., 2014). The administration of methanol leaf extract (MLE) of *O. kibbiensis* in graded doses (40, 80 and 160 mg/kg) significantly ($p<0.05$) and dose dependently reduced the number of writhes induced by acetic acid. The highest dose of the extract (160 mg/kg) had a higher percentage of inhibition compared to the standard drug, piroxicam (10 mg/kg). The intraperitoneal injection of 0.6 % acetic acid in mice evoked abdominal writhing through the release of pain mediators such as arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis (Khan *et al*., 2010; Liu *et al.,* 2020). The MLE of the *Ochna kibbiensis* might have exerted its analgesic activity in a similar pathway. In the formalin-induced pain test, the first phase is associated with the neurogenic (non-inflammatory) pain generated by the activation of nociceptors (Coelho *et al.,* 2005) while the second phase is characterized by inflammatory pain which is related to the release of prostaglandins, serotonin and plasma kinins (Shibata *et al*., 1989; Imam, *et al.,* 2012). Drugs that act primarily on the central nervous system inhibit both phases equally, while peripherally acting drugs inhibit the late phase (Shibata *et al*., 1989). In this study, the MLE of the plant significantly (at $p<0.05$) reduced pain induced by formalin during both phases with the highest dose (160 mg/kg) having higher percentage of inhibitions than the standard drug, Piroxicam (10 mg/kg) suggesting the central and peripheral activity of the plant (MLE) on both neurogenic and inflammatory pain.

Formalin-induced inflammation is caused primarily by blood leukocytes (like neutrophils) which cause increased permeability and produce oedema of the peripheral tissue and macrophages (Fantone and Ward, 1982). During the formalin-induced inflammation test, the formalin increases the paw volume compared to that of a normal rat. The MLE (at all the tested doses) of the plant significantly (at $p<0.05$) in a concentration-dependent manner reduced the paw oedema of the rat. However, in the early hours (1 – 3 h), only the highest dose, 160 mg/kg of the extract exhibited significant percentage inhibition and was also higher compared to the standard drug, piroxicam 10mg/kg, suggesting an effective onset and sustained duration of anti-inflammatory activity of the plant extract. Most NSAIDs exert their anti-inflammatory effect by inhibiting the arachidonic acid metabolism via cyclooxigenase and lipoxyganaseanzyme pathways (Liu *et al*., 2024). Thus, the MLE of *O. kibbiensis* might similarly exhibit its activity. Dvoja *et al*. (2019) reported a strong anti-inflammatory activity of the *O. schweinfurthian* extract. In addition, the analgesic and anti-inflammatory activities of the MLE might be due to the presence of phytochemical constituents and ochnaflavone reported in the leaf of the plant.

## CONCLUSION

The methanol leaf extract of *Ochna kibbiensis* has demonstrated significant analgesic and anti-inflammatory activities, rationalizing the ethnomedicinal use of the plant in the management of pain and inflammation.

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