

## Research Article

# Anti-nociceptive and anti-inflammatory activities of methanol root extract of *Andropogon gayanus* Kunth (Poaceae) in rodents

Suleiman U. Zandam <sup>a,\*</sup>, Abdullahi B. Nazifi <sup>b</sup>, Saidi Odoma <sup>c</sup>, Abdulkadir U. Zezi <sup>d</sup>, and Nuhu M. Danjuma <sup>d</sup>

<sup>a</sup> Department of Pharmacology, Federal University Dutse, Nigeria

<sup>b</sup> Department of Pharmacology and Therapeutics, Bayero University, Kano, Nigeria

<sup>c</sup> Department of Pharmacology and Therapeutics, Kogi State University, Anyigba, Nigeria

<sup>d</sup> Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria

\* **Corresponding author:** Department of Pharmacology, Federal University Dutse, PMB 7156, Dutse, Nigeria. **Tel:** +234-803-4384546; **E-mail:** [suleimanzandam@gmail.com](mailto:suleimanzandam@gmail.com)

**Background:** *Andropogon gayanus* is widely used in traditional medicine for various ailments such as postpartum pain, bronchitis and oedema.

**Objective:** This study evaluated the anti-nociceptive and anti-inflammatory activities of methanol root extract of *A. gayanus* in experimental rodents.

**Methods:** Phytochemical screening tests and acute toxicity studies were carried out. Analgesic activity using acetic acid-induced writhing response and hot plate test in mice, formalin-induced pain and carrageenan-induced paw oedema in rats were evaluated at doses of 250, 500 and 1000 mg/kg of the extract.

**Results:** Oral median lethal dose was >5000 mg/kg in both mice and rats. The extract significantly ( $p < 0.01$ ) decreased the number of writhing movements at all tested doses. It also significantly ( $p < 0.05$ ) increased the mean reaction times. A significant ( $p < 0.05$ ) decrease in mean pain scores was also observed in both phases of the formalin test at 1000 mg/kg. The extract at 1000 mg/kg significantly ( $p < 0.05$ ) reduced the oedema at the 1<sup>st</sup> hour, while at the 5<sup>th</sup> hour, all doses tested significantly reduced the oedema.

**Conclusion:** The methanol root extract of *Andropogon gayanus* possesses antinociceptive and anti-inflammatory activities.

**Keywords:** *Andropogon gayanus*, Analgesic, Anti-inflammatory, Pain

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## 1. Introduction

The importance of herbal medicines for combating various diseases has been known for ages and they are still in use worldwide (Abe and Ohtani, 2013). Indeed, medicinal plants are important in the treatment or relief

of pain and inflammation, especially in African countries where ethno-medicine is largely utilized (Mobasheri, 2012; Katanić *et al.*, 2018). Some of the important drugs used today in the management of pain and inflammatory conditions were derived from medicinal plants. Opioid analgesics for example, are alkaloids

derived from *Papaver somniferum* while non-steroidal anti-inflammatory drugs (NSAIDs) were discovered based on the structure of acetylsalicylic acid, a monoterpene from *Filipendula ulmaria* (Katanić *et al.*, 2016). Despite the achievements in technology and advancement in NSAIDs development (Altman *et al.*, 2015), these drugs still produce undesirable side effects and some of them have no value in the management of some forms of pain and inflammatory conditions (Gaskell *et al.*, 2014). Accordingly, research on medicinal plants is still required for the identification of lead compounds that may be safer and more effective in the management of pain and inflammatory conditions (Paliwal *et al.*, 2017).

*Andropogon gayanus* Kunth (Family: Poaceae) is a tufted perennial grass that is commonly known as English tambuki or gamba grass, and in Nigerian local languages as “Kalawal” (Fulfulde), “Gamba/Tsaure” (Hausa), “Eruwa ako” (Yoruba), or “Ikpo” (Igbo). It is widely used in Nigeria and other African countries as source of fodder for animals and for its various medicinal properties (Burkill, 1985). In Nigeria, the root extract is used to treat post-partum pain, cough and bronchitis, while the whole plant is used in the treatment of wounds, skin infections, hiccups, diarrhoea and gastrointestinal problems (Adjanohoun *et al.*, 1991; Etuk *et al.*, 2009). The leaves are also used for the management of swollen faces, hands and feet in Benin Republic (Verger, 1995). Despite the wide usage of this plant in ethno-medicine, there is no scientific justification to validate its use in the treatment of pain and inflammatory conditions. This study therefore was carried out to investigate the anti-nociceptive and anti-inflammatory activities of the methanol root extract of *A. gayanus* using experimental animal models.

## 2. Methods

### 2.1 Test drugs and chemicals

The test drugs used were acetylsalicylic acid (ASA, Aspirin®) and morphine (Martindale Pharmaceuticals, U.K.), while the chemicals used were acetic acid (Ranbaxy Laboratories Ltd, India), Methanol, 10 %<sub>v/v</sub> Formalin solution and Carrageenan (Sigma-Aldrich Chemie GmbH, Germany).

### 2.2 Laboratory animals

Wistar rats and Swiss albino mice of both sexes (120-180 g and 18-24 g respectively) were obtained from the Animal Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were allowed free access to standard feed and water *ad libitum*. They were kept in clean cages bedded with saw dust which was replaced every three days. All experiments performed were in accordance with Ahmadu Bello University Research Policy (Revised 2010) and the experimental protocols were approved by the University Animal Ethics Committee (Approval no: DAC/IW-OT/015-43348).

### 2.3 Plant material

The fresh roots of *A. gayanus* were collected in August, 2015 at Samaru, Sabon Gari Local Government Area of Kaduna state, Nigeria. The plant was identified and

authenticated by Mallam Namadi Sanusi, a botanist in the Herbarium of the Department of Botany, Ahmadu Bello University, Zaria. A voucher number 247 was assigned by comparing with a previously deposited voucher specimen.

### 2.4 Preparation of the plant extract

The collected roots were dried under shade for two weeks and size reduced with a mortar and pestle. The powdered roots (2 kg) was extracted by cold maceration with 2 liters of aqueous-methanol (in the proportion of 30:70) for 48 hours. The extract obtained was evaporated to dryness in an evaporator under reduced pressure and controlled temperature (40-50°C). Thereafter, the extract was weighted and preserved in a desiccator until needed for further studies. Aqueous solutions were freshly prepared for each study using distilled water.

### 2.5 Phytochemical screening

Preliminary phytochemical screening tests were carried out on *A. gayanus* extract to detect the presence or absence of secondary metabolites (alkaloids, saponins, tannins, flavonoids, terpenoids, anthraquinones, glycosides and cardiac glycosides) using standard methods (Evans, 2002).

### 2.6 Acute toxicity testing

The median lethal dose (LD<sub>50</sub>) of the extract was determined using Lorke's method (1983) through the oral route. The study was carried out in two phases in both mice and rats. In phase 1, three groups of three animals each were administered *A. gayanus* extract orally in geometrically increasing doses (10, 100 and 1000 mg/kg).

The treated animals were observed for four hours post administration for signs of toxicity and for 24 hours for mortality. After 24 hours, phase 2 was initiated and three animals were given specific doses of the extract orally (1600, 2900 and 5000 mg/kg). The animals were then observed for signs of toxicity for the first 4 hours and mortality for 24 hours. The geometric mean of the lowest lethal dose and the highest non-lethal dose was evaluated as the LD<sub>50</sub> of the extract. The same procedure was repeated in both species of animals using the intraperitoneal routes.

### 2.7 Groups and treatments

The experimental animals used in each model were assigned into five groups with each group containing six animals (n=6). The first group served as negative control and was administered distilled water (1 ml/kg and 10 ml/kg in rats and mice respectively). The second, third and fourth groups were treated with methanol root extract of *A. gayanus* at doses of 250, 500 and 1000 mg/kg respectively, while the fifth group served as positive control and were treated with either 300 mg/kg of ASA (in acetic acid and anti-inflammatory tests) or 10 mg/kg of morphine (in hot plate and formalin tests).

All drugs were administered orally (*p.o.*).

## 2.8 Anti-nociceptive tests

### Writhing test in mice

Acetic acid induced writhing method as described by Koster *et al.*, (1959) was adopted for evaluation of analgesic activity. Writhing is defined as a characteristic stretching behaviour, extension of hind legs, twist of the body or contraction of the abdomen of mice so that the abdomen touches the floor (Mishra *et al.*, 2011). Thirty mice of both sexes were divided into five groups and treated as previously described. Sixty minutes after treatment, the mice received 0.6% acetic acid (10 ml/kg) intraperitoneally to induce pain. Five minutes after acetic acid injection, the animals were placed individually in an observation box, and observed for 10 minutes. The number of writhes produced by each mouse within the 10 minutes period was counted. A reduction in the number of writhes as compared to the control animals was considered as evidence of analgesia and expressed as percent inhibition of writhes.

### Hot plate (thermal sensitivity) test in mice

This method was carried out as described by Eddy and Leimback (1953). The paws of mice are very sensitive to heat at temperatures which are not damaging to the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The temperature of the hot plate was set at  $45 \pm 1^\circ\text{C}$  (Mishra *et al.*, 2011). Thirty mice were fasted 12 hours prior to the experiment. The mice were divided into five groups and treated as previously described. Thereafter, the animals were individually placed on the hot plate and the time until either licking or jumping occurred (reaction time) was recorded using a stop-watch, 60, 90, 120 and 150 minutes after treatment.

### Formalin test in rats

The method described by Dubuisson and Dennis (1977) as modified by Murray *et al.*, (1988) was adopted in this experiment. Thirty rats were divided into 5 groups of 6 rats each and treated as previously described. One hour after the administrations, 50  $\mu\text{l}$  of freshly prepared 2.5 % formalin was injected subcutaneously into the plantar surface of the left hind paw of each rat. The observation was made in two phases, phase 1 (neurogenic pain) and phase 2 (inflammatory pain). Phase 1 lasts for 5 minutes from the time of formalin administration while phase 2 covers from 15 minutes to 60 minutes after formalin administration with 10 minutes lag period between the two phases. The severity of pain was monitored based on the following scale: Score 0 (rat stands firmly or walks on the injected paw), Score 1 (injected paw partially elevated or favoured), Score 2 (injected paw is clearly lifted off the floor), Score 3 (rat licks, chews or shakes the injected paw).

### 2.9 Carrageenan induced rat paw oedema test for anti-inflammatory activity

The anti-inflammatory study was carried out using the method described by Winter *et al.*, (1962). Thirty rats were assigned into five groups of six rats each and were also treated as previously described. Sixty minutes after administration of the various agents, oedema was

induced by injecting 0.1 ml carrageenan solution (0.1% w/v) into the planter side of the left hind paw of each rat. The paw sizes were then measured with digital vernier caliper at 0, 1, 2, 3, 4, and 5 hours after the carrageenan injection (Sharma *et al.*, 2010).

### 2.10 Data analyses

Data obtained for acetic acid test was analyzed by one way analysis of variance (ANOVA) followed by Dunnett *post hoc* test. Data for hot plate and carrageenan tests were analyzed using repeated measures ANOVA followed by Bonferroni *post hoc* test for multiple comparison, while data for formalin test was analyzed using non-parametric Kruskal-Wallis test. The results were considered significant at  $p \leq 0.05$  and were expressed as mean  $\pm$  standard error of mean (S.E.M).

## 3. Results

The methanol root extract of *A. gayanus* obtained was a sticky sweet smelling solid with greenish-black colour. The percentage yield of the extract was 1.8 % w/w. Preliminary phytochemical screening revealed the presence of glycosides, saponins, triterpenes, tannins, flavonoids and alkaloids.

The mice and rats that received *A. gayanus* extract orally did not show signs and symptoms of toxicity after the first four hours of administration and no death was recorded in both phases of the experiment. The oral median lethal doses for both mice and rats were greater than 5000 mg/kg body weight.

Administration of methanol root extract of *A. gayanus* significantly ( $p < 0.01$ ) decreased the number of writhes caused by acetic acid in a non-dose-dependent manner. Out of the three dose levels of the extract tested, the highest percentage inhibition of writhes (59.5%) was obtained at 500 mg/kg, and the effect of the extract at all doses tested was less than that of the standard (ASA) at 300 mg/kg (**Table 1**).

The methanol root extract of *A. gayanus* significantly ( $p < 0.05$ ) increased the mean reaction time at different doses. The peak of activity was recorded at 120 min where all doses of the extract tested were able to significantly ( $p < 0.05$ ) increase the reaction time when compared to control. Morphine 10 mg/kg, significantly ( $p < 0.05$ ) increased the reaction time at all-time levels except at 150 min (**Figure 1**).

During both phases of formalin test, *A. gayanus* extract at 250 and 500 mg/kg showed insignificant reduction in pain response. Morphine in phase 1 and 2 significantly ( $p < 0.01$  and  $p < 0.001$  respectively) reduced the severity of pain when compared to the negative control. Similarly, the extract at 1000 mg/kg significantly ( $p < 0.05$  and  $p < 0.01$ ) decreased pain severity in phase 1 and phase 2 respectively (**Table 2**).

*A. gayanus* extract at 1000 mg/kg and ASA (300 mg/kg) significantly ( $p < 0.05$ ) reduced inflammation at the 1st hour when compared to distilled water group. At the 5th hour, the extract at all doses tested and ASA significantly ( $p < 0.01$ ) reduced inflammation (**Figure 2**). The effect of *A. gayanus* extract was evaluated over time by comparing the 3rd hour (which marks the peak of

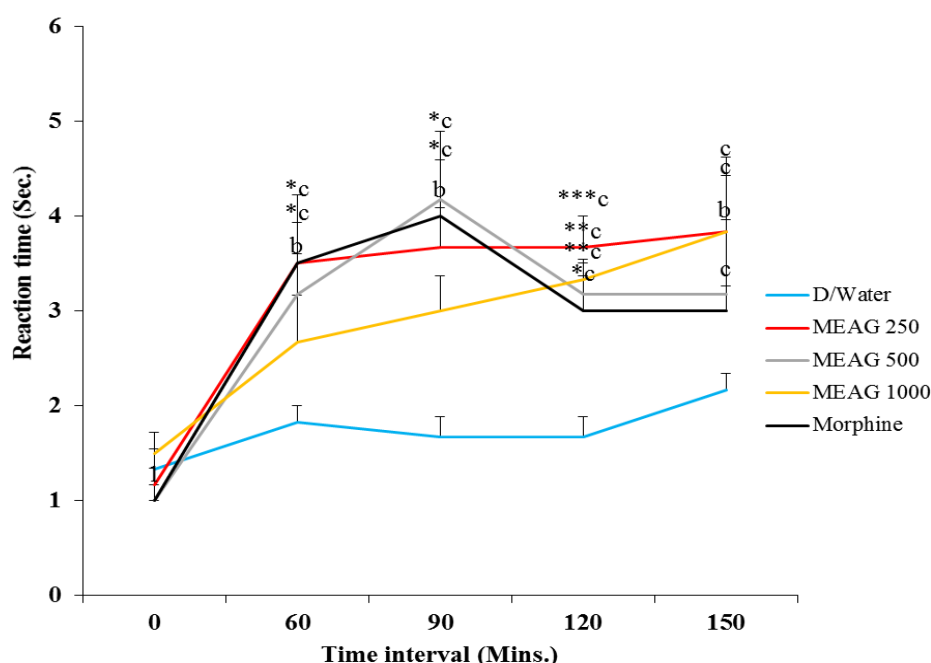
inflammation due to carrageenan) to other times. The extract at 250 mg/kg significantly ( $p < 0.01$ ) decreased paw oedema in rats at the 1st hour, while at 500 mg/kg,

a significant reduction in paw oedema was observed at the 1st and 2nd hour when compared to the 3rd hour.

**Table 1:** Effect of methanol root extract of *Andropogon gayanus* on acetic acid induced writhing in mice

Treatment (mg/kg)	Number of writhes Mean $\pm$ SD	Percentage Inhibition (%)
D/Water (10 ml/kg)	28.00 $\pm$ 2.21	-
MEAG (250)	14.67 $\pm$ 2.62**	47.6
MEAG (500)	11.33 $\pm$ 2.06***	59.5
MEAG (1000)	13.67 $\pm$ 2.56***	51.2
ASA (300)	6.33 $\pm$ 0.84***	77.4

Values are Mean  $\pm$  S.E.M., \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$  compared to Distilled water (D/Water) group – One way ANOVA followed by Dunnett test, n=6, ASA = Acetylsalicylic acid, MEAG = Methanol root extract of *Andropogon gayanus*



**Figure 1:** Effect of methanol root extract of *Andropogon gayanus* on thermally-induced pain in mice.

Values are Mean  $\pm$  SEM, \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$  compared to Distilled water (D/Water) group, a, b, and c =  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  respectively compared to reaction time 0 – Repeated measures ANOVA followed by Bonferroni test; n=6, MEAG = Methanol root extract of *Andropogon gayanus*.

#### 4.0 Discussion

Phytochemical screening of *A. gayanus* extract revealed the presence of some secondary metabolites known to possess anti-nociceptive and anti-inflammatory activities (Abubakar *et al.*, 2016). Saponins possess anti-nociceptive, anti-inflammatory and anti-allergic activities (Akkol *et al.*, 2007; Yassin *et al.*, 2013), while alkaloids have been reported to exhibit anti-inflammatory and antioxidant activities (Arrau *et al.*, 2010; Singh *et al.*, 2010). Tannins are also used in the treatments of cuts and wounds, haemorrhoids, catarrh, heavy menstrual flows and inflammatory conditions of the digestive tract (Evans, 2002). Thus, the

antinociceptive and anti-inflammatory effects exhibited by *A. gayanus* root extract may be due to its phytoconstituents either individually or in combination.

LD<sub>50</sub> determination of plants used in ethno-medicine is very important because it provides useful information regarding their margin of safety. In the acute toxicity studies, the oral LD<sub>50</sub> of *A. gayanus* suggest that it is practically non-toxic (Loomis and Hayes, 1996) in both rats and mice because there were no signs of toxicity or death in both phases of the studies.

The acetic acid-induced writhing test is the most commonly used method for screening agents with

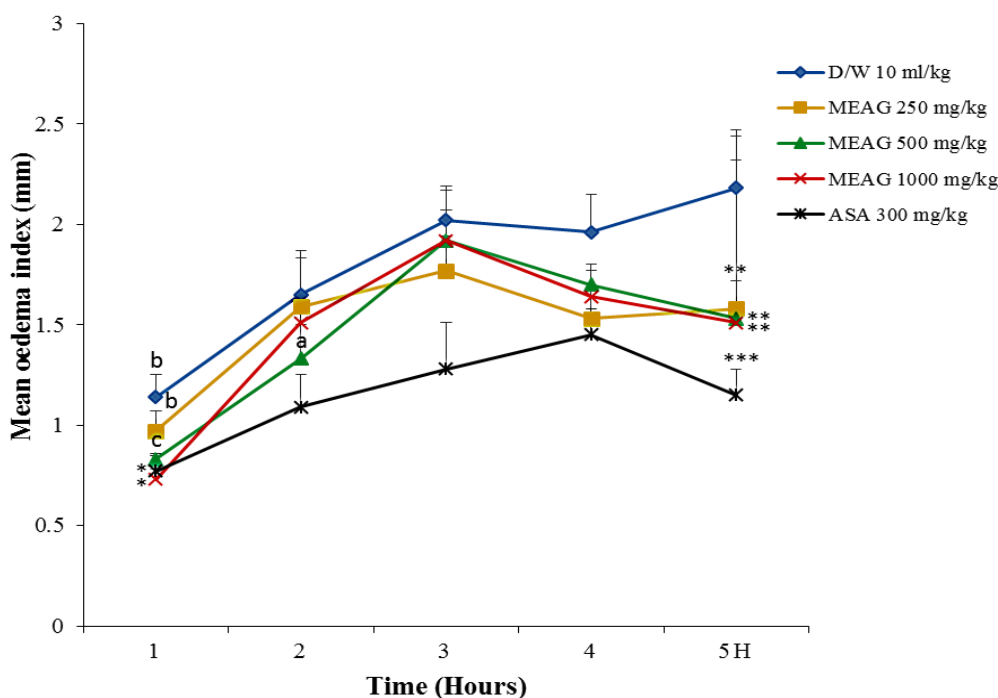
peripheral analgesic activity in mice although it is sensitive to both centrally and peripherally acting analgesics (Vogel, 2008). The intra-abdominal injection of acetic acid leads to the release of pain mediators such as prostaglandin and cytokines which may be responsible for the induced pain (Ikeda *et al.*, 2001).

Indeed, NSAIDs such as ASA and piroxicam inhibit cyclooxygenase (COX) enzyme and the synthesis of prostaglandins (Immer *et al.*, 2003). In this study, the significant inhibition of acetic acid-induced abdominal writhes by *A. gyanus* extract suggests analgesic activity which may be due to peripheral effects.

**Table 2:** Effect of methanol root extract of *A. gyanus* on formalin-induced pain in rats

Treatment (mg/kg)	Mean Pain Scores	
	Phase 1	Phase 2
D/Water (1 ml/kg)	3.00 ± 0.00	3.00 ± 0.00
MEAG (250)	2.17 ± 0.48	2.17 ± 0.54
MEAG (500)	2.50 ± 0.22	2.50 ± 0.22
MEAG (1000)	2.00 ± 0.23*	1.50 ± 0.22**
Morphine (10)	1.67 ± 0.33**	0.83 ± 0.31***

Values are Mean ± S.E.M., \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$  compared to Distilled water (D/Water) group – Kruskal-wallis test. n = 6, MEAG = Methanol root extract of *Andropogon gyanus*



**Figure 2:** Effect of methanol root extract of *Andropogon gyanus* on carrageenan-induced rat paw oedema.

Values are Mean ± S.E.M., \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$  compared to Distilled water (D/W) group; a, b, and c =  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  respectively compared to time 3 hr – Repeated measures ANOVA followed by Bonferroni- test, n = 6, MEAG = Methanol root extract of *Andropogon gyanus*, ASA = Acetyl salicylic acid

Administration of *A. gyanus* extract caused a prolonged latency period, indicating an increase in the nociceptive threshold observed in the thermal test of nociception used in this study. The hot plate model has been used as an experimental model to measure nociception, especially for the evaluation of centrally acting analgesic drugs (Vogel, 2008). The test model produces paw licking and jumping behavioural responses, both of which are considered supraspinally integrated

responses (Chapman *et al.*, 1985; Pavin *et al.*, 2011). Studies have shown that opioid analgesics like morphine, tramadol and pentazocine produce centrally mediated analgesic responses by interacting with the opioid receptors (Furst and Hosztafi, 2008; Pavin *et al.*, 2011; Gholami *et al.*, 2015). Thus, the prolongation of the reaction time by *A. gyanus* root extract at all doses tested suggests it also possesses centrally mediated analgesic properties.

In the formalin test, *A. gyanus* extract (at 1000 mg/kg) significantly decreased the pain responses in both phases of the test although it was more effective in reducing pain in the second phase. The extract was less efficacious than morphine, which significantly inhibited the two phases of the test. The formalin test is considered the most valid model for the clinical evaluation of pain by provoking a biphasic response (Huskaar and Hole, 1987; Tjølsen *et al.*, 1992, Fischer *et al.*, 2014). The first phase results from the direct chemical stimulation of formalin on the nociceptive afferent fibers, principally the C-fibers, and the release of substance P and bradykinin (Huskaar and Hole, 1987; Tjølsen *et al.*, 1992). Pain responses in this phase is inhibited by centrally acting analgesics (da Rocha *et al.*, 2012). On the other hand, the second phase is associated with the development of an inflammatory response (with the release of algescic mediators such as prostaglandins) and an increase in synaptic transmission in the spinal cord (Tjølsen *et al.*, 1992). The pain responses in this phase can be inhibited by both peripherally and centrally acting analgesics (Munro, 2009; da Rocha *et al.*, 2012). Therefore, the findings from this study further showed that *A. gyanus* extract is effective in promoting both peripherally and centrally mediated analgesia.

The carrageenan test is an established model for investigating anti-inflammatory properties of new drug therapies and is highly sensitive to NSAIDs (Denadai-Souza *et al.*, 2009). The formation of oedema caused by carrageenan is in two phases; the first hour after carrageenan injection (first or early phase), involves the release of serotonin, histamine and bradykinin while the second or late phase (2-5 hours) with increased oedema formation that remains up to the fifth hour involves the release of prostaglandins (Xu *et al.*, 2014). The second phase of swelling involves not only the elevated production of prostaglandins, but also attributed to the induction of inducible COX-2 in the hind paw (Nantel *et al.*, 1999; Abdelwahab *et al.*, 2015). The results obtained from this study showed that oedema induced by carrageenan was inhibited by both *A. gyanus* extract and the ASA during the 5 hrs of the studies, although, some of the inhibitions were not statistically significant. Significant inhibition came up at the first hour only with the highest dose of the extract and with all the tested doses at the fifth hour.

## 5.0 Conclusion

The methanol root extract of *Andropogon gyanus* Kunth possesses anti-nociceptive and anti-inflammatory activities. This supports its ethno-medical uses as analgesic and anti-inflammatory agent.

## Conflict of Interest declaration

The authors declare no conflict of interest.

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