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Antipyretic, Antiinflammatory and Antinociceptive Activities of Aqueous Bark Extract of *Acacia Nilotica* (L.) Delile in Albino Mice

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Abstract

Acacia nilotica has been used to manage several diseases including pain, inflammation and fever. However, its efficacy has not been scientifically validated. The aim of this study therefore is to investigate the antinociceptive, antipyretic and anti-inflammatory activities of its aqueous extracts. The plant extract was collected from Loita division, Narok county in Kenya. A total of 96 albino mice with an average weight of 20 g was used for this study. Antinociceptive activity was determined by use of formalin−induced writhing test. A writhe was recorded by a stopwatch following the stretching of the abdomen and/or stretching of at least one hind limb. Anti-inflammatory activity was established by a formalin induced inflammation test. Hourly changes in paw sizes and reduction of edema around the paw was determined using a venier calipers. Antipyretic activity was carried out using Brewer's yeast induced pyrexia. Temperature of each mouse was determined rectally by thermal probe thermometer. The aqueous leaf extracts of *A. nilotica* reduced pain, inflammation and fever mostly at dose 150 mg/kg body weight. Based on these findings it was concluded that the present study has demonstrated the antinociceptive, anti-inflammatory and antipyretic potential of aqueous leaf extracts of *A. nilotica* in albino mice and will serve as good bio-resource for generating readily available herbal formulations that are more effective in the treatment of pain, inflammation and fever conditions which are cheaper than the conventional synthetic drugs and have no side effects.

Keywords: *Acacia nilotica*; Pyrexia; Antinociceptive activity; Antiinflammatory activity

Introduction

Synthetic chemicals have for many years, been effectively used for the treatment of many illnesses [1]. Traditional plant-derived compounds have also been used as medicine since ancient history, playing an important role in health care, especially in the rural settings where access to modern medicine is limited [2-5]. Plants have been shown to contain phytochemicals (bioactive compounds) that act as defense systems to combat various diseases [1]. Numerous studies have reported success in validation of medicinal plants towards treatment of various diseases [6-9]. For instance, plant species such as *Acanthus hirsutus* [10], *Aegiceras corniculatum* [11] and *Pongamia pinnata* [12] have been reported for their anti−nociceptive, anti-inflammatory and antipyretic activities respectively. *A. nilotica* L (family Mimosaceae) is a multipurpose tree that grows to a height of 20 m [13]. The tree is a subtropical species spread throughout Asia, Africa and America and is an integral part of rural and agro pastoral systems in these regions [14]. It has been reported that different parts of the plant contain various compounds that can be harnessed to provide therapeutic ingredients against diseases [15,16]. Rural populations have used the barks of the plant as an anthelmintic, aphrodisiac and antidiuretic agent. Furthermore, illnesses such as wounds, leprosy and skin diseases have also been shown to be treated by the bark exudates of *A. nilotica* [17,18]. *A. nilotica* roots have been effective in treating tumors, cancer and tuberculosis as reported by [19]. Leaves and the gum from this tree have also been shown to be effective antibacterial agents and success has been achieved in their use against diarrhea, dressing of wounds and inflammation [14,19,20]. The medicinal properties of this tree are attributed to the presence and abundance of chemical substances such as alkaloids, phenols, flavonoids and steroids that aid in the healing processes [15,21]. Despite these numerous reports on the effectiveness of *A. nilotica* against a vast array of diseases, data on specific disease symptoms such as inflammation, pyrexia and nociception is scarce.

Materials and Methods

Collection and preparation of plant materials

Fresh bark plant material of *A. nilotica* was collected from Loita division, Narok county in Kenya. This plant is believed by the locals to have medicinal value against wounds and diabetes. The plant material was identified and authenticated with help from the Department of Botany, Kenyatta University. Preparation of plant extract was carried out using a protocol as described by Nostro et al. [22]. The powdered materials were kept at room temperature away from direct sunlight in closed dry khaki paper bags.

Extraction

The powdered material was separately extracted with single distilled water at 125 g/L on a 60^{0}C water bath for 6 hours. The solvent extract was then concentrated to dryness under reduced pressure and the residue preserved at 4°C for future use. Exactly 400 g of *A. nilotica* was dissolved in 3.2L of single distilled water in a conical flask and the mixture put on the water bath. Decantation and filtration processes through a No.1 Whatman filter paper were repeated until the sample became clear. The filtrate was freeze-dried, weighed and stored in an airtight plastic bag and refrigerated until it was used for bioassay. This procedure gave 20 g of freeze-dried *Acacia nioltica*.

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Preparation of reagents and extracts used for bioassay

The plant extract for determination of antinociceptive, anti-inflammatory and antipyretic activities were prepared in the following manner (Tables 1-3).

Animal models

Swiss albino mice of average weight of 20 g were used in this study. These animals were maintained in the experimental room at the Animal House, Department of Biochemistry and Biotechnology, Kenyatta University. The room was set at controlled conditions of 25 ± 2°C temperature, 55% humidity and 12 hr light/12 hr darkness photoperiod regime to acclimatize the animals. The mice were kept in a cage and fed with standard laboratory food and water *ad libitum*.

Group	Status	Treatment
I	Control	Normal saline (0.1 ml) + Formalin (0.05 ml of 2.5% formalin)
II	Baseline	Formalin (0.05 ml of 2.5% formalin)
III	Standard	Diclofenac (12 µl of 75 mg/3 ml diclofenac sodium + 0.1 ml Normal saline) + Formalin (0.05 ml of 2.5% formalin)
IV	Test-1	50 mg/kg extract (0.001 g + 0.1 ml Normal saline) + Formalin (0.05 ml of 2.5% formalin)
V	Test-2	100 mg/kg extract (0.002 g + 0.1 ml Normal saline) + Formalin (0.05 ml of 2.5% formalin)
VI	Test-3	150 mg/kg extract (0.003 g + 0.1 ml Normal saline) + Formalin (0.05 ml of 2.5% formalin)

Table 1: Treatment protocol for the determination of antinociceptive activity for the aqueous extract of the *A. nilotica*.

Group	Status	Treatment
I	Control	Normal saline (0.1 ml) + Formalin (0.05 ml of 2.5% formalin)
II	Baseline	Formalin (0.05 ml of 2.5% formalin) + Formalin (0.05 ml of 2.5% formalin)
III	Standard	Diclofenac (10 µl of 75 mg/3 ml diclofenac sodium + 0.1 ml Normal saline) + Formalin (0.05 ml of 2.5% formalin)
IV	Test-1	50 mg/kg extract (0.001 g + 0.1 ml Normal saline) + Formalin (0.05 ml of 2.5% formalin)
V	Test-2	100 mg/kg extract (0.002 g + 0.1 ml Normal saline) + Formalin (0.05 ml of 2.5% formalin)
VI	Test-3	150 mg/kg extract (0.003 g + 0.1 ml Normal saline) + Formalin (0.05 ml of 2.5% formalin)

Table 2: Treatment protocol for the determination of anti-inflammatory activity for the aqueous bark extract of *A. nilotica*.

Group	Status	Treatment
I	Control	Yeast (0.03 g of 10 ml/kg of 15% w/v yeast + 0.2 Normal saline) + Normal saline (0.1 ml)
II	Baseline	Yeast (0.03 g of 10 ml/kg of 15% w/v yeast + 0.2 Normal saline)
III	Standard	Yeast (0.03 g of 10 ml/kg of 15% w/v yeast + 0.2 Normal saline) + Paracetamol (0.286 mg in 0.1 ml normal saline)
IV	Test-1	Yeast (0.03 g of 10 ml/kg of 15% w/v yeast + 0.2 Normal saline) + 50 mg/kg extract (0.001 g + 0.1 ml Normal saline)
V	Test-2	Yeast (0.03 g of 10 ml/kg of 15% w/v yeast + 0.2 Normal saline) + 100 mg/kg extract (0.002 g + 0.1 ml Normal saline)
VI	Test-3	Yeast (0.03 g of 10 ml/kg of 15% w/v yeast + 0.2 Normal saline) + 150 mg/kg extract (0.003 g + 0.1 ml Normal saline)

Table 3: Treatment protocol for the determination of antipyretic activity for the aqueous bark extract of *A. nilotica*.

Experimental Design

Determination of antinociceptive activity

To determine the antinociceptive activity of the plant extract, a formalin–induced writhing test was carried out using a method described by Wheeler-Aceto et al. [23]. Groups of 5 mice each was as test and control specimen (Table 1).

Determination of anti-inflammatory activity

To determine the anti-inflammatory effect of the extract in mice, a formalin induced inflammation test was carried out as described by Hosseinzadeh and Younesi [6]. Inflammation was induced by intraperitoneal injection of 0.05 ml of 2.5% formalin into the left hind paw of each mouse (Table 2). Hourly changes in paw sizes and reduction of edema around the paw was determined using a venier calipers.

Determination of antipyretic activity

The antipyretic activity of the plant extract was evaluated using Brewer's yeast induced pyrexia as described by Loux et al. [24]. According to the protocol, 15% aqueous suspension of Brewer's yeast was first prepared using normal saline (Table 3).

Results

The results showed that the aqueous exctract of *A. nilotica* had some antinociceptive activity against formalin-induced nociception, which was indicated by reduction in paw-licking time compared to the control (Figure 1 and Table 4). The administration of diclofenac sodium before injection of formalin significantly reduced pain in the acute phase and was not statistically different from plant extract at dose 50 mg/kg body weight (p<0.05; Table 4). Results of study showed that the

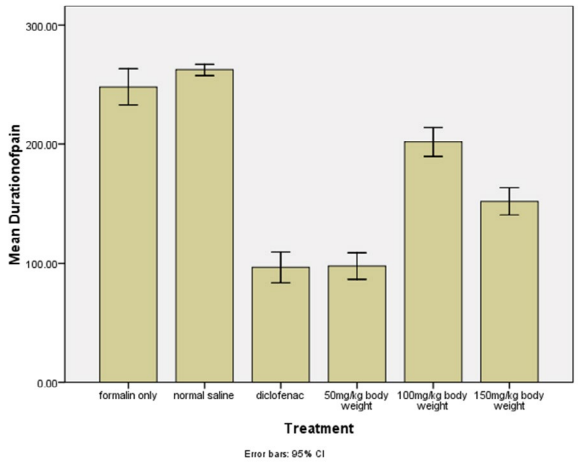

Figure 1: Antinociceptive effect of *A. nilotica* aqueous extract on acute pain.

Group	Treatment	Mean paw-licking time(sec) ± SD
1 Control	Normal saline	262.4 ± 3.84^{d}
2 Baseline	Formalin	248.0 ± 12.20^{d}
3 Standard	Diclofenac	96.4 ± 10.35^{a}
4 Test-1	50 mg/kg	97.4 ± 8.96^{a}
5 Test-2	100 mg/kg	201.8 ± 9.90^{c}
6 Test-3	150 mg/kg	152.0 ± 9.27^{b}

Mean values ± SD with the same letters are not statistically different from one another by ANOVA followed by Tukey's post hoc test (p>.05). n=5.

Table 4: Antinociceptive effect of *A. nilotica* aqueous extract on acute pain.

dose of 50 mg/kg aqueous bark extract of *A. nilotica* exerts significant antinociceptive effect ($p<0.05$) characterized by decreasing duration of licking and biting time in the acute or first phase (0-5 min) of formalin induced pain. This analgesic effect was statistically different from the control ($p<0.05$; Table 4). However, at a dose of 100 mg/kg plant extract, the duration of pain response increased significantly and it was statistically different from the control ($p<0.05$; Table 4). Dose of 150 mg/kg plant extract significantly reduced paw licking time compared to the control ($p<0.05$; Table 4). In this study, aqueous bark extract of *A. nilotica* showed a significant antinociceptive activity on the late phase of formalin induced pain though not in a dose dependent manner (Figure 2 and Table 5). Diclofenac (reference drug) did lower chronic pain significantly compared to the control ($p<0.05$; Table 5). The plant extract at dose of 50 mg/kg body weight had a significant decrease in paw licking time compared to the baseline and control ($p<0.05$; Table 5). Treatment of mice with leaf extracts of *A. nilotica* showed some anti-inflammatory activity against formalin-induced edema, which was indicated by reduction in paw edema (Figure 3 and Table 6). In the first hour, the group of mice treated with plant extract at dose of 100 mg/kg body weight showed greater inhibition of inflammation and this was indicated by a reduction in paw diameter to 81.73% compared to the other dose levels and the reference drug (Figure 3 and Table 6). Aqueous bark extract of *A. nilotica* showed anti-inflammatory effect though not in a dose dependent manner compared to the control ($p>0.05$; Table 6). In the second hour, all mice treated with the leaf extracts of *A. nilotica* at doses of 50, 100 and 150 mg/kg body weight recorded a reduction of inflammation to 83.88%, 76.46% and 80.07% respectively (Table 6). Although, the anti-inflammatory effectiveness was not in a dose dependent manner, the extract at dose level of 100 mg/kg body weight was better comparable to reference drug, diclofenac and the control ($p>0.05$; Table 6). In the third hour, the anti-inflammatory

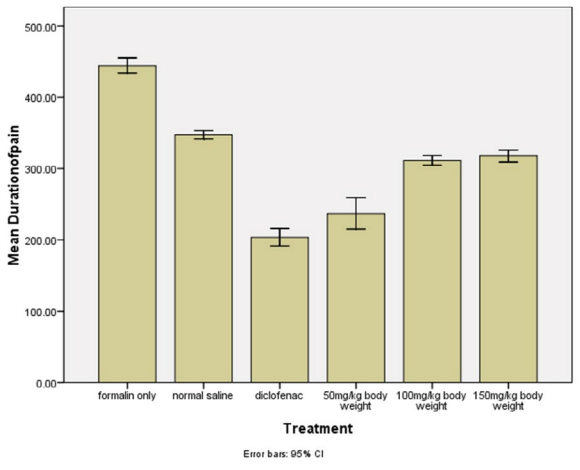

Figure 2: Antinociceptive effect of *A. nilotica* aqueous extract on chronic pain.

Group	Treatment	Mean paw-licking time(sec) ± SD
1 Control	Normal saline	347.4 ± 4.39^{d}
2 Baseline	Formalin	444.4 ± 8.50^{e}
3 Standard	Diclofenac	203.6 ± 9.86^{a}
4 Test-1	50 mg/kg	237.0 ± 17.88^{b}
5 Test-2	100 mg/kg	311.4 ± 5.36^{c}
6 Test-3	150 mg/kg	317.6 ± 6.80^{c}

Mean values ± SD with the same letters are not statistically different from one another by ANOVA followed by Tukey's post hoc test ($p>0.05$) n=5.

Table 5: Antinociceptive effect of *A. nilotica* aqueous extract on chronic pain.

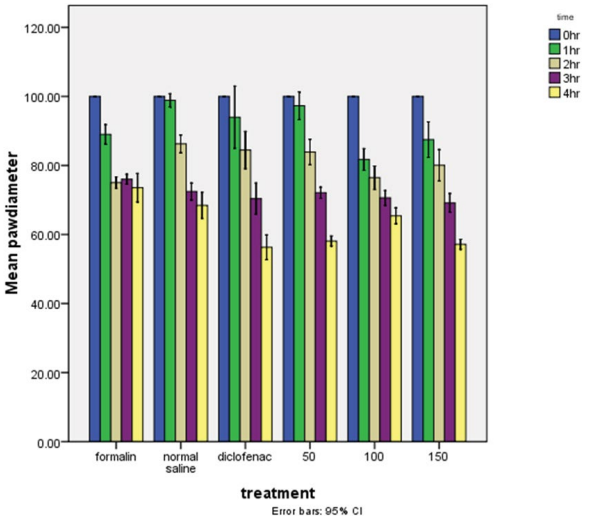

Figure 3: Anti-inflammatory effect of *A. nilotica* aqueous extract on albino mice.

Group	Treatment	Percent change in paw diameter (mm) after drug administration				
		0 hr	1 hr	2 hr	3 hr	4 hr
Control	Normal saline	100.00 ± 0.00^{Ed}	98.87 ± 1.54^{Dd}	86.24 ± 2.06^{Cd}	72.44 ± 1.98^{Bd}	68.42 ± 3.04^{Ad}
Baseline	Formalin	100.00 ± 0.00^{Ec}	88.98 ± 2.28^{Dc}	75.01 ± 1.32^{Cc}	76.00 ± 1.14^{Bc}	73.54 ± 3.32^{Ac}
Standard	Diclofenac	100.00 ± 0.00^{Ebc}	93.96 ± 7.23^{Dbc}	84.45 ± 4.35^{Cbc}	70.38 ± 3.62^{Bbc}	56.30 ± 2.9^{Abc}
Test-1	50 mg/kg	100.00 ± 0.00^{Ec}	97.29 ± 3.51^{Dc}	83.88 ± 2.94^{Cc}	72.08 ± 1.30^{Bc}	58.10 ± 1.17^{Ac}
Test-2	100 mg/kg	100.00 ± 0.00^{Eab}	81.73 ± 2.53^{Dab}	76.46 ± 2.71^{Cab}	70.61 ± 1.71^{Bab}	65.40 ± 1.87^{Aab}
Test-3	150 mg/kg	100.00 ± 0.00^{Ea}	87.48 ± 4.14^{Da}	80.07 ± 4.20^{Ca}	69.15 ± 2.18^{Ba}	57.16 ± 1.15^{Aa}

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another by ANOVA followed by Tukey's post hoc test ($p>0.05$) n=5.

Table 6: Anti-inflammatory effect of *A. nilotica* aqueous extract on albino mice.

activity of the plant extract was in a dose dependent manner. The anti-inflammatory properties of aqueous leaf extracts of *A. nilotica* at dose of 50 mg/kg and 100 mg/kg body weight was comparable to reference drug. At this hour, the mice treated with 150 mg/kg of the herbal extract exhibited the highest anti-inflammatory effect to 69.15% (Table 6). Four hours after drug administration, *A. nilotica* at all dose levels (50, 100 and 150 mg/kg body weight) was found to lower the formalin-induced inflammation (Table 6). The group of mice treated with 150 mg/kg body weight inhibited inflammation to 57.16% which was close to the reference drug which showed inflammatory inhibition by 58.10% (Table 6). Treatment of mice with leaf extracts of *A. nilotica* showed some antipyretic activity against brewer's yeast induced pyrexia, which was indicated by reduction in rectal temperature (Figure 4 and Table 7). In the first hour after treatment, plant extract at dose of 50 mg/kg body weight showed the highest antipyretic activity among the extract dosages by reducing fever to 99.07% (Figure 4 and Table 7). Aqueous bark extract of *A. nilotica* exhibited antipyretic activities but not in a dose dependent manner (Table 7). In the second hour, plant extract at dose of 150 mg/kg body weight showed the highest effectiveness in reducing the rectal temperature to 98.89% compared to the reference

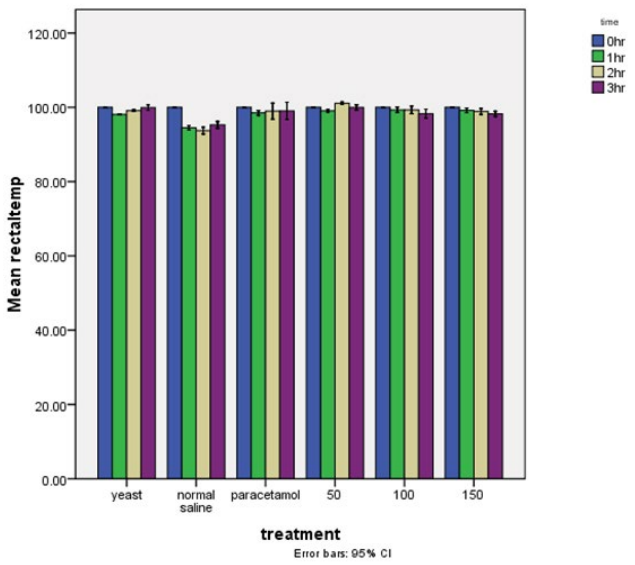

Figure 4: Antipyretic effect of *A. nilotica* aqueous extract on albino mice.

Group	Treatment	Percent change in rectal temperature (°C) after drug administration			
		0 hr	1 hr	2 hr	3 hr
Control	Normal saline	100.0 ± 0.00^{Ba}	94.49 ± 0.55^{Aa}	93.70 ± 0.84^{Aa}	95.28 ± 0.85^{Aa}
Baseline	Yeast	100.0 ± 0.00^{Bb}	98.10 ± 0.01^{Ab}	99.09 ± 0.21^{Ab}	99.91 ± 0.70^{Ab}
Standard	Paracetamol	100.0 ± 0.00^{Bb}	98.50 ± 0.61^{Ab}	98.96 ± 2.04^{Ab}	99.03 ± 2.20^{Ab}
Test-1	50 mg/kg	100.0 ± 0.00^{Bc}	99.07 ± 0.32^{Ac}	101.10 ± 0.31^{Ac}	99.95 ± 0.66^{Ac}
Test-2	100 mg/kg	100.0 ± 0.00^{Bb}	99.30 ± 0.69^{Ab}	99.30 ± 0.96^{Ab}	98.29 ± 1.14^{Ab}
Test-3	150 mg/kg	100.0 ± 0.00^{Bb}	99.16 ± 0.56^{Ab}	98.89 ± 0.73^{Ab}	98.23 ± 0.65^{Ab}

Mean values ± SD with the same capital letters down the columns and same small letters across the rows are not statistically different from one another by ANOVA followed by Tukey's post hoc test (p>0.05) n=6.

Table 7: Antipyretic effect of *A. nilotica* aqueous extract on albino mice.

drug. *A. nilotica* had an antipyretic effect in a dose dependent way (Figure 4 and Table 7). Plant extract at dose 50 mg/kg showed a pyretic effect instead as the rectal temperature was increased at this hour to 101.10% (Table 7). In the third hour, plant extract at dose 150 mg/kg was more effective as shown by a reduction of fever to 98.23% compared to the plant extract at the other dosages and reference drug, diclofenac. The antipyretic effect of the herbal medicine on fever was in a dose dependent manner (Figure 4 and Table 7).

Discussion

The search for bioactive components which can be used as non-conventional analgesics, NSAIDs and antipyretics has received considerable attention in recent times because of the increasing worldwide development of lasting solutions to pain, inflammation and fever which are safe to human and with no side effects as seen with modern medicine. Thus, this study was oriented to evaluate the curative capacity of aqueous bark extract of *A. nilotica* against pain, inflammation and fever. The evaluation of antinociceptive, anti-inflammatory and antipyretic properties of the leaf extract was done by formalin induced pain and inflammation and brewer's yeast induced pyrexia in albino mice. Subcutaneous injection of a dilute aqueous formalin (formaldehyde) solution into the dorsal surface of the rat or mouse hind paw elicits two distinct quantifiable nociceptive behaviors, i.e. flinching/shaking and licking/biting of the injected paw [25,26]. This formalin-induced nociceptive behavior shows an early and a late phase. The early phase, which starts immediately following injection of formalin, only lasts approximately 5 min and is probably due to direct chemical stimulation of nociceptors (acute pain). The second phase, which lasts 20 to 40 min, starts approximately 15 to 30 min following formalin injection and experimental data suggest that peripheral, inflammatory processes are involved [27]. The formalin test differs from most other nociceptive tests, such as the hot plate, tail flick and tail pinch tests, in that it enables evaluation of analgesic activity towards moderate, continuous pain generated by injured tissue. As a result, it has been suggested that this test provides a more valid model been suggested that this test provides a more valid model such as the hot plate and tail flinch tests [25,28,29]. The two distinct phases in formalin test are due to direct effect of formalin on nociception and due to inflammation with the release of serotonin, histamine, bradykinin and prostaglandins and at least to some degree, the sensitization of central nociceptive neurons [25,26,30]. Stimulation of opioid receptors has also been suggested as a possible mechanism of action against neurogenic pain [31]. In this study, aqueous leaf extracts of *A. nilotica* showed a significant antinociceptive effect by reducing the formalin-induced paw licking time in both phases. The highest analgesic effect was at 50 mg/kg dose level for both acute and chronic pain. These findings suggest both direct analgesic effects on the nociceptor blockage and an inhibition of the synthesis and/or release of inflammatory pain mediators such as prostaglandins. These results are similar to other previous studies on evaluation of antinociceptive activities of medicinal plant extracts. That the aqueous extracts of *A. nilotica* demonstrated a reduction in the formalin-induced paw licking time in both phases is consistent with [32] who observed antinociceptive activity of hydroalcoholic extract of *Marrubium parviflorum* against formalin-induced pain in mice. Similarly, the methanolic leaf extract of *Securinega virosa* demonstrated related antinociceptive effect in acetic acid induced writhing test and formalin test models [33]. That the aqueous extracts of *A. nilotica* produced non-dose dependent analgesic activity is related to studies by Zarei et al. [34] who observed the antinociceptive activities of *Melissa officinalis* leaf extracts in laboratory animals. The dose ranges used in this study were within the dose ranges used by Ishola et al. [35-37]. The aqueous bark extract of *A. nilotica* showed the highest analgesic effect at lower dose of 50 mg/kg body weight in early and late phases. This may be due to the fact that the high dose takes longer to be absorbed across the peritoneum cavity.

The antinociceptive effect of *A. nilotica* can be attributed to one or more groups of the phytoconstituents observed in the extracts. Several studies have shown the antinociceptive activity of such compounds. Phytochemical screening of methanolic leaf extract of *Securinaga virosa* revealed the presence of flavonoids, saponins, tannins, glycosides, alkaloids and steroids [33]. A study on the phytochemical composition of *A. nilotica*, has revealed presence of saponins, tannins, flavonoids, alkaloids and phenols [38]. Analgesic and anti-inflammatory effects have been observed in flavonoids as well as tannins [39]. Flavonoids such as quercetin are known to be effective in acute inflammation [40]. There are also reports on the analgesics effects of alkaloids, essential oils and saponins [41-43]. The analgesic and anti-inflammatory effect of the extracts in this study may therefore, be due to the presence of flavonoids, tannins, alkaloid or saponins. Flavonoids are widely shown to target prostaglandins which are involved in the pain perception

through moderating opioidergic mechanism. These findings strongly recommend that this medicinal plant has peripheral analgesic activity and their mechanisms of action may be mediated through inhibition of local peritoneal receptors which may be the involvement of cyclooxygenase inhibition potential. The profound analgesic activity of this medicinal plant may be due to the interference of their active principle(s) with the release of pain mediators. Tissue damage and injury are always associated with pain and inflammation.

In this formalin test, the mice used were treated with several treatments to reduce inflammation. Formalin test is a biphasic response where first phase is the direct effect of formalin which involves neurogenic pain. The pain is usually initiated when harmful mechanical, thermal or chemical stimuli agitate the peripheral terminals of particular main afferent neuron named nociceptors [44]. The second phase is involved in the inflammatory reactions. In this study, it was noticed that exposure of formalin induced inflammation to various treatments resulted in a significant inhibition of inflammation. The aqueous extracts of *A. nilotica* was found to significantly suppress the inflammation when treated with different concentrations. After five hours of the test period, the aqueous bark extract of *A. nilotica* produced appreciable anti-inflammatory activity against formalin induced inflammation in albino mice. A dose level of 100 mg/kg and 150 mg/kg body weight showed the highest anti-inflammatory activity. Lower dose of 50 mg/kg was not as effective and may be explained by the fast metabolism, clearance and inactivation of the lower concentration of the active principles or the lower dose was an insufficient concentration of the active principles.

The association of both antinociceptive activity and moderate anti-inflammatory effect observed with the extracts has also been shown in non-steroidal anti-inflammatory drugs (NSAIDs). It is a well-established fact that NSAIDs exert their analgesic and anti-inflammatory activity by the inhibition of cyclo-oxygenase activity [45]. The anti-inflammatory effects of the extracts may be due to their content of flavonoids, tannins, alkaloids and saponins. Several studies have shown the antinociceptive activity of such compounds. A study by Muhammad et al. [46] showed that the *Viola betonicifolia* methanolic extract was found to contain alkaloids, saponins, flavonoids, tannins, proteins, and phenolic compounds where the anti-inflammatory activity of *V. betonicifolia* was attributed to these groups of chemical compounds. The antiinflammtory effect of the medicinal plant extract of *A. nilotica* was not evident in every concentration of the extracts as early as the first hour of formalin injection but maximum inhibition was during the fifth hour. It did not maintain the suppression of the inhibition throughout the duration of the study. These findings could have been due to the fact that the active principles in the extracts required biotransformation so as to have an anti-inflammatory effect.

Brewer's yeast was used to induce fever in albino mice. Fever was recorded 19 hrs after yeast injection since yeast takes a total of about 19 hrs to cause the elevation of body temperature [47]. Subcutaneous injection of Brewer's yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful test for the screening of plants materials as well as synthetic drugs for their antipyretic effect [48,49]. Yeast induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins [50]. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclo-oxygenase enzyme activity. There are several mediators for pyrexia and the inhibitions of these mediators are responsible for the antipyretic effect [51].

The oral administration of *A. nilotica* significantly attenuated rectal temperature of yeast induced albino mice. Thus it can be postulated that *A. nilotica* contained pharmacologically active principle(s) that interfere with the release of prostaglandins. After three hours of the test period, the aqueous bark extract of *A. nilotica* produced appreciable antipyretic activity against brewer's yeast induced pyrexia in albino mice. Dose of 150 mg/kg body weight demonstrated the greatest rectal temperature lowering activity. This finding was in agreement with the effects of other medicinal plants in laboratory animals. Similar work carried out by Okokon and Nwafor [52] showed that the hydro alcoholic extract of *Rosa alba* plant possessed a significant antipyretic effect in yeast induced elevation of body temperature in experimental rats. It was revealed that the extract showed dose dependent antipyretic activity. At a dose of 200 mg/kg it showed significant antipyretic activity.

Non-steroidal anti-inflammatory drugs produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus. Work done by showed that the antipyretic activity of hydro alcoholic extract of *Rosa alba* is probably by inhibition of prostaglandin synthesis in hypothalamus. Therefore it is possible that the antipyretic action of aqueous extracts of *A. nilotica* was related to the inhibition of prostaglandin synthesis in hypothalamus. However, other alternative mechanisms for blocking fever cannot be ruled out. Further hydro alcoholic extract of *Rosa alba* was found to contain carbohydrates, alkaloids, glycosides, flavonoids and tannins, through preliminary photochemical screening. Qualitative phytochemical screening in this study revealed that the aqueous bark extract of *A. nilotica* contain tannins, saponins, phenolics, alkaloids and flavonoids. A number of these phytochemicals have been shown to exhibit inhibitory action on cyclooxygenase enzyme and, as a result, produce antipyretic activity by preventing the formation of prostaglandins or by increasing the concentration of body's own antipyretic components [52]. Flavonoids are known to target prostaglandins which are involved in the pyrexia. Hence the presence of flavonoids in the aqueous bark extract of *A. nilotica* plant may be contributory to its antipyretic activity. The presence of alkaloids in this extract could also be responsible for the antipyretic activity. For instance, according to Reanmongkol et al. [53] while evaluating on antipyretic effects of alkaloids extracted from the stem bark of *Hunteria zeylanica,* reported that alkaloids also possesses antipyretic effects. The antipyretic activity of the aqueous bark extract of *A. nilotica* may also be attributed to the presence of saponins, which are involved in inhibition of prostaglandin synthesis. According to the study of Zakaria et al. [54] saponins are suggested to act synergistically to exert antipyretic activity. In a related study, the antipyretic effect of ethanolic root extracts of *Asparagus racemosus* on yeast-induced hyperthermia in rats was attributed to the saponins in the extracts [55]. It was observed that aqueous bark extract of *A. nilotica* at lower dose levels of 50 and 100 mg/kg body weight were not as effective as the higher dose of 150 mg/kg body weight, and thus may be explained by the fast metabolism, clearance and inactivation of the lower concentration of the active principles. It's also likely that at the lower dose there is simply not a sufficient concentration of the active principle(s).

The aqueous bark extract of *A. nilotica* at all the dose levels did not lower rectal temperature in the first and second hours as effectively as in the third hour. These findings could have been due to the fact that the active principles in the extracts required biotransformation so as to become antipyretic. That the dose level of 150 mg/kg body weight of the aqueous bark extract of *A. nilotica* was marginally effective than paracetamol, suggests a possible better blockage of prostaglandins biosynthesis or mimicry of paracetamol action by the active principles

in the extracts. It is also possible that the herbal extracts were efficiently inhibiting alternative mechanisms for blocking fever. The decline in rectal temperature in case of treatment with the medicinal plants extracts was not as sudden as that of paracetamol administration. Therefore, the extracts offer some advantage over the standard drug (paracetamol).

Conclusion

In conclusion, the present study has demonstrated the antinociceptive, anti-inflammatory and antipyretic potential of aqueous leaf extracts of *A. nilotica* in albino mice. The aqueous bark extract of *A. nilotica* was able to inhibit pain sensation of both phases. It is, therefore, possible to find opioid analgesics as well as analgesics in aqueous leaf extracts of *A. nilotica* that act by inhibition of inflammatory pathways responsible for pain. Furthermore, the classes of phytochemicals in aqueous bark extract of *A. nilotica* has previously been observed to contribute to antipyretic and antinociceptive activities. The aqueous leaf extracts of *A. nilotica* has potent anti-inflammatory activity in rats in a dose dependent manner. The mechanism of anti-inflammation by aqueous bark extract of *A. nilotica* might be related with the compounds of bioactive and phytochemicals present in the plants. Therefore, these medicinal plants have the prospect to be used as herbal remedy for inflammation. The significant reduction in pyrexia in mice when treated with standard drugs as well as different doses of extracts, reflect that aqueous bark extract of *A. nilotica* is endowed with potent antipyretic properties.

It is also evident from the study that the antipyretic activity of aqueous leaf extracts of *A. nilotica* at 150 mg/kg body weight was more effective compared to other doses used in this study. Therefore, the aqueous bark extract of *A. nilotica* might help in preventing pain, inflammation and fever complications and serve as good bio-resource for generating a readily available herbal formulation that is more effective in the treatment of pain, inflammation and fever conditions which is cheaper than the conventional synthetic drugs and has no side effects. However, the modes of antinociceptive, anti-inflammatory and antipyretic actions of the studied extracts are still obscure. The present study, therefore, scientifically confirms and supports the traditional use of aqueous leaf extracts of *A. nilotica* for management of fever, inflammation and painful conditions.

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Conflict of Interest

The authors declared no conflict of interest.

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