Anti-pyretic, Anti-inflammatory and Analgesic Activities of Aqueous Stem Extract of Cynanchum Viminale (L.) in Albino Mice

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Abstract

Cynanchum viminale 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 analgesic, 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 were used for this study. Analgesic activity was determined by use of formalin–induced writhing test. A write 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 callipers. 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 C. viminale 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 analgesic, anti-inflammatory and antipyretic potential of aqueous leaf extracts of C. viminale 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.

Keywords: Cynanchum viminale; Pyrexia; Anti-nociceptive activity; Anti-inflammatory activity

Introduction

Herb- and plant-derived medicines have been used since ancient times and considered as part of our health remedies. The tendency of using natural products for the treatment of serious life-threatening diseases [1-5] has been increasing. It is stated that natural products are easily biodegradable, possess least environmental hazards, represent minimal side effects and are available at affordable prices. Although most of medicinal activities of the plants have been well-documented, the others are yet to be verified [6]. C. viminalis (family asclepiadaceae) is a perennial shrub that originated from Madagascar and grows in the Acacia savanna in semi-arid habitats of Kenya, Uganda, Tanzania and Somalia where it is comparatively widespread [7]. C. viminalis subspecies include cconsinica, another subspecific taxon in the C. viminalis complex, is described as new based on morphological, ecological and molecular evidence. C. viminalis subspecies cconsinica occurs at altitudes of around sea level close to shore up to the higher foot of Mt. Kilimanjaro. Typically it grows in Acacia savanna and scrub of semi-arid to arid habitats in Tanzania, Kenya, Uganda, and Somalia. There is less information on the analgesic, anti-inflammatory and antipyretic properties of this plant and that's why this research was believed to cover the gap on studies about its use as herbal medicine to manage these ailments.

Materials and Methods

Collection and preparation of plant materials

Fresh stem material of C. viminalis 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 [8]. 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°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 375 g of C. viminalis was dissolved in 3 L 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 44 g of freeze-dried C. viminalis.

Preparation of reagents and extracts used for bioassay

The plant extract for determination of analgesic, anti-inflammatory and antipyretic activities were prepared in the following manner (Table 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.

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Received November 30, 2016; Accepted February 09, 2016; Published February 11, 2016


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Table 1: Treatment protocol for the determination of analgesic activity for the aqueous stem extract of C. viminale

<table>
<thead>
<tr>
<th>Group</th>
<th>Status</th>
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</tr>
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<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>Normal saline (0.1 ml)+Formalin (0.05 ml of 2.5% formalin)</td>
</tr>
<tr>
<td>II</td>
<td>Baseline</td>
<td>Formalin (0.05 ml of 2.5% formalin)+Formalin (0.05 ml of 2.5% formalin)</td>
</tr>
<tr>
<td>III</td>
<td>Standard</td>
<td>Diclofenac (10 µl of 75 mg/3 ml diclofenac sodium+0.1 ml Normal saline)+Formalin (0.05 ml of 2.5% formalin)</td>
</tr>
<tr>
<td>IV</td>
<td>Test-1</td>
<td>50 mg/kg extract (0.001 g+0.1 ml Normal saline)+Formalin (0.05 ml of 2.5% formalin)</td>
</tr>
<tr>
<td>V</td>
<td>Test-2</td>
<td>100 mg/kg extract (0.002 g+0.1ml Normal saline)+Formalin (0.05 ml of 2.5% formalin)</td>
</tr>
<tr>
<td>VI</td>
<td>Test-3</td>
<td>150 mg/kg extract (0.003 g+0.1 ml Normal saline)+Formalin (0.05 ml of 2.5% formalin)</td>
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Table 2: Treatment protocol for the determination of anti-inflammatory activity for the aqueous stem extract of C. viminale

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Table 3: Treatment protocol for the determination of antipyretic activity for the aqueous stem extract of C. viminale

Experimental design

Determination of analgesic activity: To determine the analgesic activity of the plant extract, a formalin--induced writhing test was carried out using a method described by [9]. Groups of 5 mice each were as test and control specimen (Table 1). The mice were individually placed in a glass beaker and observed for writhing. The number of stretches per animal was recorded for the following 30 minutes. A writhing was recorded following the stretching of the abdomen and/or stretching of at least one hind limb according to [10].

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 [10]. 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 [11]. According to the protocol, 15% aqueous suspension of Brewer’s yeast was first prepared using normal saline (Table 3). Temperatures of each mouse was then determined by thermal probe thermometer rectally at hourly interval for three hours after extract and drug administration.

Results

This study showed that the aqueous stem extract of C. viminale exhibited an analgesic activity against the first phase of formalin induced pain in mice though not in a dose dependent manner (Figure 1 and Table 4). There was a significant analgesic activity at dose of 100 mg/kg body weight as it was seen instead by decreased paw licking time. Diclofenac lowered the pain significantly compared to the plant extracts at all dose levels (p<0.05; Table 4). Aqueous stem extract of C. viminale showed analgesic activity against chronic pain though not in a dose dependent manner (Figure 2 and Table 5). Analgesic effectiveness of the leaf extracts at the dose level of 150 mg/kg body weight was better compared to the other dose levels (p<0.05; Table 5) and from the control. These dose levels however were not as effective as diclofenac (reference drug). C. viminale exhibited anti-inflammatory activity against formalin-induced edema in albino mice (Figure 3 and Table 6). In the first hour after drug administration, plant extract at dose of 100 mg/kg body weight showed the highest inhibition of inflammation by 87.42% among the extract dosages and diclofenac (Table 6). Aqueous extract of C. viminale exhibited anti-inflammatory activities but not in a dose dependent manner (Figure 3 and Table 6). In the second hour, plant extract at dose of 50 mg/kg body weight showed the highest inhibition of inflammation by 75.09% among all the treatment groups. C. viminale had anti-inflammatory effect though not in a dose dependent way (Figure 3 and Table 6). In the third hour, plant extract at dose 50 mg/kg was more effective by reduction of paw diameter by 66.84% compared to diclofenac.
compared to the other treatment groups (Table 6). In the fourth hour, the effectiveness of diclofenac was compared to the plant at dosages of 100 mg/kg and 150 mg/kg body weight though plant extract at dose 100 mg/kg was found to reduce inflammation better by 53.13% (Table 6). Treatment of mice with leaf extracts of *C. viminale* 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 150 mg/kg body weight showed the highest antipyretic activity among the extract dosages by reducing rectal temperatures to 94.08% (Table 7). Aqueous extract of *C. viminale* exhibited antipyretic activities but not in a dose dependent manner and was seen to be better in reducing fever at dose 50 mg/kg body weight than the reference drug (Figure 4 and Table 7). In the second hour, plant extract at dose of 100 mg/kg body weight showed the highest effectiveness in reducing the rectal temperature to 96.91% compared to the reference drug. *C. viminale* exhibited an antipyretic effect in a non-dose dependent way (Figure 4 and Table 7). Plant extract at dose 50 mg/kg and 150 mg/kg body weight showed a pyretic effect instead as the rectal temperature was increased to 100.52% (Table 7). In the third hour, plant extract at dose 150 mg/kg was more effective compared to the reference drug as fever was reduced to 96.29% (Table 7). The antipyretic effect of the herbal medicine on fever was in a dose
against formalin-induced pain. Paracetamol at 100 mg/kg 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 analgesic effect of C. viminale can be attributed to one or more groups of the phytoconstituents observed in the extracts. Several studies have shown the analgesic activity of such compounds.

Discussion

This study was oriented to evaluate the curative capacity of aqueous stem extract of C. viminale against pain, inflammation and fever. Evaluation of analgesic, 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 [12,13]. 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 and experimental data suggest that peripheral, inflammatory processes are involved [14]. 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 [12,15,16]. 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 [9,13,17]. Stimulation of opioid receptors has also been suggested as a possible mechanism of action against neurogenic pain [18]. Aqueous stem extract of C. viminale showed the highest analgesic effect at dose 100 mg/kg and also 50 mg/kg for acute pain but a dose of 150 mg/kg had the least paw licking time indicating better analgesic effect. 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 analgesic activities of medicinal plant extracts. That the aqueous extracts of C. viminale demonstrated a reduction in the formalin-induced paw licking time in both phases is consistent with [19] who observed analgesic activity of hydroalcoholic extract of Marrubium pavuliferum against formalin-induced pain in mice. Similarly, the methanolic leaf extract of Securinaga virosa demonstrated related analgesic effect in acetic acid induced writhing test and formalin test models [20]. That the aqueous stem extract of C. viminale produced non-dose dependent analgesic activity is related to studies by [21] who observed the analgesic activities of Melissa officinalis leaf extracts in laboratory animals. The dose ranges used in this study were within the dose ranges used by [22-24]. The aqueous stem extract of C. viminale 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 analgesic effect of C. viminale can be attributed to one or more groups of the phytoconstituents observed in the extracts. Several studies have shown the analgesic activity of such compounds. Physiochemical screening of methanolic leaf extract of Securinaga virosa revealed the presence of flavonoids, saponins, tannins, glycosides, alkaloids and steroids [20]. A study on the phytocomposition of C. viminale has revealed presence of saponins, tannins, flavonoids, alkaloids and phenols [25]. Analgesic and anti-inflammatory effects have been observed in flavonoids as well as tannins [26]. Flavonoids such as quercetin are known to be effective in acute inflammation [27]. There are also reports on the anxieties effects of alkaloids, essential oils and saponins [28-30]. 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 these medicinal plants have 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 these medicinal plants 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 [31].

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 stem extract of C. viminale was found to significantly suppress the inflammation when treated with different concentrations. After five hours of the test period, the aqueous stem extract of C. viminale exhibited greater anti-inflammatory activity at dose 150 mg/kg. 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 analgesic 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-

<table>
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<th>Group</th>
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<th>Percent change in rectal temperature (°C) after drug administration</th>
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<td></td>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>Baseline</td>
<td>Normal saline</td>
<td>100.0 ± 0.000a</td>
</tr>
<tr>
<td></td>
<td>Yeast</td>
<td>100.0 ± 0.000a</td>
</tr>
<tr>
<td>Standard</td>
<td>Paracetamol</td>
<td>100.0 ± 0.000a</td>
</tr>
<tr>
<td>Test-1</td>
<td>50 mg/kg</td>
<td>100.0 ± 0.000a</td>
</tr>
<tr>
<td>Test-2</td>
<td>100 mg/kg</td>
<td>100.0 ± 0.000a</td>
</tr>
<tr>
<td>Test-3</td>
<td>150 mg/kg</td>
<td>100.0 ± 0.000a</td>
</tr>
</tbody>
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Table 7: Antipyretic effect of C. viminale aqueous extract on albino mice.

inflammatory activity by the inhibition of cyclo-oxygenase activity [32]. The anti-inflammatory effects of the extracts may be due to their content of flavonoids, tannins, alkaloids and saponins. Several studies have shown the analgesic activity of such compounds. A study by [33] 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 anti-inflammatory activity of the four medicinal plants extracts 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. They 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 [34]. 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 [35,36]. Yeast induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins [37]. 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 [38]. The oral administration of C. viminale significantly attenuated rectal temperature of yeast induced albino mice. Thus it can be postulated that C. viminale contained pharmacologically active principle(s) that interfere with the release of prostaglandins. After three hours of the test period, the aqueous stem extract of C. viminale 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. These findings were in agreement with the effects of other medicinal plants in laboratory animals. Similar work carried out by [39] 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. 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 photochemical screening in this study revealed that the aqueous extracts of C. viminale contain tannins, saponins, phenolics, alkaloids and flavonoids. A number of these photochemicals 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 [40]. Flavonoids are known to target prostaglandins which are involved in the pyrexia. Hence the presence of flavonoids in the aqueous stem extract of C. viminale 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 [41] 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 stem extract of C. viminale may also be attributed to the presence of saponins, which are involved in inhibition of prostaglandin synthesis. According to the study of [42] 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 [43].

It was observed that aqueous stem extract of C. viminale 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 stem extract of C. viminale 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 leaf extracts of C. viminale was marginally effective than paracetamol, suggests a possibly better blockage of prostaglandins biosynthesis or mimicry of paracetamol action by the active principles in the extract. It is also possible that the herbal extract was efficiently inhibiting alternative mechanisms for blocking fever. The decline in rectal temperature in case of treatment with C. viminale was not as sudden as that of paracetamol administration. Therefore, the extract offers some advantage over the standard drug (paracetamol).

Conclusion
In conclusion, the present study has demonstrated the analgesic, anti-inflammatory and antipyretic potential of aqueous leaf extracts of C. viminale in albino mice. The aqueous leaf extracts of C. viminale 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 C. viminale that act by inhibition of inflammatory pathways responsible for pain. Furthermore, the classes of phytochemicals in aqueous leaf extracts of C. viminale have previously been observed to contribute to antipyretic and analgesic activities. The aqueous leaf extracts of C. viminale has potent anti-inflammatory activity in mice in a non-dose dependent manner. The mechanism of anti-inflammation by aqueous leaf extracts C. viminale might be related with the compounds of bioactive and phytochemicals present in the plants. Therefore, C. viminale has 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 stem extract of C. viminale is endowed with potent antipyretic properties. It is also evident from the study that the antipyretic activity of aqueous leaf extracts of C. viminale at 150 mg/kg body weight was more effective compared to other doses used in this study. Therefore, the aqueous stem extract of C. viminale might help in preventing pain, inflammation and fever. It may serve as good bio-resource for generating a readily available herbal formulation that is more effective, cheaper than the conventional synthetic drugs and has no side effects. However, the modes of analgesic, anti-inflammatory and antipyretic actions of the studied extract are still obscure. The present study, therefore, scientifically confirms and supports the traditional use of aqueous stem extract of C. viminale for management of fever, inflammation and pain.

Acknowledgments
The authors acknowledge Mr. James Adino and Mr. Wycliffe Wenwa for their technical support.

Conflict of Interest
The authors declared no conflict of interest.

Funding
This study received no funding.

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