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Analgesic activity of aqueous extract of *Vernonia hymenolepis* (A. Rich) a traditional medicine plant used in Kenya for toothache

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Abstract

The main aim of the study was to ascertain the analgesic properties of *Vernonia hymenolepis* leaves to validate its use for the treatment of toothache. The plant is widely used as a traditional herb by communities in Trans Nzoia County, Kenya for treatment of various infections including toothache. However its efficacy has not been established. Leaves of the plant were collected from Trans Nzoia County, Kenya and identified at University of Nairobi Herbarium. An aqueous extraction of leaves was prepared. Formalin test was carried out using 30 male albino wister mice to determine antinociceptive effect and the painful response at 0 – 10 min (Early) and 15 – 60 min (late phase). Acetylsalicylate at dose of 100 mg/Kg was used as a positive control. The dose significantly ($p<0.05$) reduced the time spent in pain behavior in both phases hence indicating that the plant posses antinociceptive activity. It's concluded that *Vernonia hymenolepis* possesses analgesic property.

Keywords: *Vernonia hymenolepis*, Analgesic, Anti-inflammatory, Antinociceptive.

Introduction

Vernonia hymenolepis belongs to the family of Asteraceae and has about 1000 species of forbs and shrubs. Some species are known as ironweed. It has numerous distinct subgenera and subsections.[1] It is an ever green shrub that grows up to 5 meters high. The stem has spines and its green in color, the leaves are leathery pubescent and tomentose beneath. The pyllaries are white, pink or purple while the flowers are mauve white. It is a leafy indigenous vegetable that is commonly cultivated by farmers in Nigeria and Cameroon.[2] *Vernonia hymenolepis* also occurs along rivers, roadsides, in forest margins, old cultivation areas, in bushed grassland and also in montane forest.

Its leaves are consumed fresh and in dry form as garnish, potherb or salad. It is used to cure pneumonia, hypertension and also to heal and stop bleeding wounds. Juice from its crashed leaves is used to treat diarrhea in babies and jaundice.[3] Studies have been documented on its use in treatment of malaria, typhoid, Amoebiasis and abdominal conditions like constipation in communities in Kenya.[4] It's widely used traditionally by Herbalist and communities in Trans Nzoia County, Kenya in treatment of toothache. However, the pain alleviating properties of these species have not been explored and documented. The aim of this study was to ascertain and validate the use of *Vernonia hymenolepis* extract for antinociceptive activity using formalin test.

Materials and Methods

Plant collection, identification and extraction

Leaves of *Vernonia hymenolepis* were collected from Trans Nzoia County, Kenya and identified in the Department of Botany, University of Nairobi and voucher specimens deposited (RO2011/001). The plant Leaves was shade dried under the shade and milled into powder using a molly grinder. The powder was then packed in a clean air tight polythene papers. One hundred grams of the plant material (Leaves) was soaked in one liter of distilled water for 72 hours in a conical flask. Filtration was done using Whatman No.1 filter paper and the filtrate collected in a beaker and then covered using aluminum foil. The filtrate was then freeze dried and yielded 32% of dry powder. The extracts were placed in airtight bottle and stored in a refrigerator at 4ºC awaiting subsequent bioassays.

Experimental animals

Thirty Male albino mice (Wister) aged 6-8 weeks and weighing 25-30 grams were used for formalin test. Food and water were given ad libitum. Animals were allowed 7 days for acclimatization. They were kept at temperature of 22ºC to 25°C and relative humidity of 50%. Diurnal rhythms were regulated with a twelve hour light: twelve hour dark cycle. Each mice was used only once in the experiment. The Pain experiments were performed in conformity to the guidelines issued by the International Association for the Study of Pain for animal pain experimentation.[5]

Analgesic activity

Formalin test and Sensorimotor Activity Testing

The mice were randomly assigned to three groups of six mice each and were intraperitoneally administered 0.5ml each of 100 mg/kg of aqueous extract of *Vernonia hymenolepis* (Group 1), Physiological saline (Group 2) and acetylsalicylic acid (Group 3) were used as negative and positive control respectively. Twenty micro liters (20 µl) of 1% formalin was injected intradermally on the plantar surface of the hind paw of each mouse one hour after administration of the test extract and also the controls. The time in seconds spent in paw licking as an index of painful response was determined at 0 – 10 min (Early) and 15– 60 min (late phase) after formalin injection. The data was presented as Mean ± S.E.M of time(s) spent in pain behaviour. The mean of time (s) spent in pain behaviour for the extract was compared with that of the control.

The pull-up test was performed in order to confirm that the Antinociceptive activity observed in the extract was independent of muscle relaxant and sensorimotor retardation effects.[6] The mice were fully extended in an inverted position one hour after administration of extract/control. The end point of the experiment was set when the mouse in attempting to gain an upright position by touching the hand or fingers of the experimenter with both forepaws simultaneously.[7] The end point was recorded using a stop watch, while the cut-off point of experiment was set at fifteen seconds.

Statistical analysis

The data was analyzed using one-way analysis of Variance (ANOVA) test and subsequently subjected to turkey post hoc test for multiple comparisons. $p<0.05$ were considered statistically significant.

Results

At 100 mg/kg b.w.t, the extract was significantly ($p<0.05$) antinociceptive compared to the normal physiologiacal saline. The extract had comparable antinociceptive effects compared with acetylsalicylic acid at a dose of 100 mg/kg b.w.t in both phases of formalin test. It caused a reduction in the duration of time spent in pain behaviour with the early phase ($p< 0.0005$, 56.92 ± 7.013s versus. 143.75± 11.9s negative control) and late phases ($p< 0.01$, 32.33± 1.97s versus. 84±3.19s negative control). Figure 1 and 2 shows the antinociceptive activities of both early phase and late phase. The late phase of the extract showed better analgesic effects compared to the positive control (Acetylsalicylic acid). There is a significant difference between the negative controls and the extracts.

Table 1: Shows the summary of means and standard error of mean of acetylsalicylic acid, aqueous extract and Physiological saline of the time spent in seconds in behaviour in formalin test.

Test sample	Mean and Standard error of mean (Early phase)	Mean and Standard error of mean (Late phase)
Acetylsalicylic acid(100mg/kg)	31.67±9.67 ***	44.85±6.99*
Vernonia hymenolepis aqueous extract(100mg/kg)	56.92±7.013***	32.33±1.97***
Physiological saline	143.75±11.9	84±3.19

*p< 0.05; *** = p< 0.0005 Compared to control; n=6

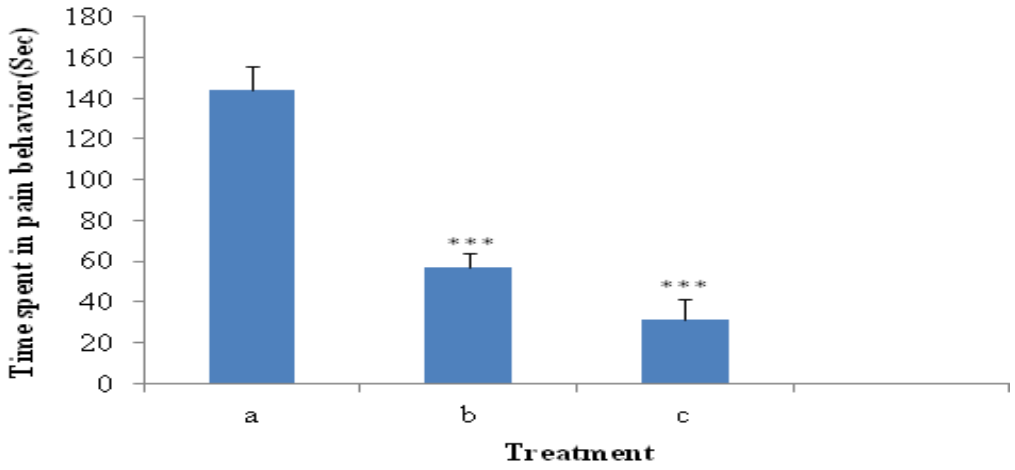

Figure 1: Effect of the intraperitoneal administration of 100 mg/kg of aqueous leaf extract of *Vernonia hymenolepis* in the formalin test in early phase. Key: *** = p< 0.0005; a = (physiological saline); b =*Vernonia hymenolepis* aqueous extract; c = Acetylsalicylic acid.

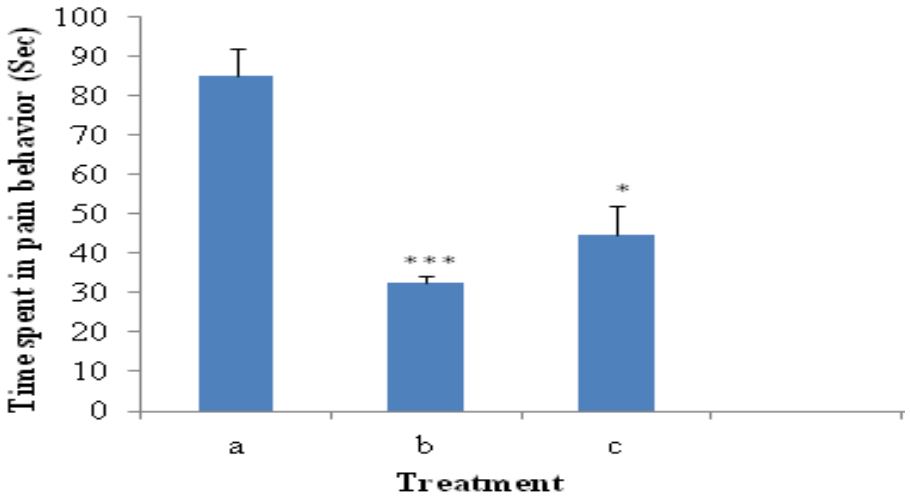

Figure 2: Effect of the intraperitoneal administration of 100 mg/kg of aqueous leaf extract of *Vernonia hymenolepis* in the formalin test in late phase. Key: * = p< 0.01, *** = p< 0.0005; a=Control (physiological saline); b= *Vernonia hymenolepis* aqueous extract; c=Acetylsalicylic acid.

Discussion

In this study aqueous extracts of *Vernonia hymenolepis* leaves showed significant antinociceptive activity in both phases of formalin test. The plant caused reduction in the duration of time spent in pain behavior for aqueous extract in both the early phase (56.92 ± 7.013s versus. 143.75± 11.9s control) and late phases (32.33± 1.97s versus. 84±3.19s control). The result implies that the use of plant leaves by herbalists for treatment of toothache is justified. Other studies by various authors have shown that leaves of various species of Vernonia posses' analgesic properties such as *Vernonia lasiopus*[8], *Vernonia guineensis*[9], *Vernonia venosa*[10] and *Vernonia cinerea*[11].*Vernonia aurilulifera* leaves are used in Cameroon to treat cataract and in Ethiopia to treat.[12, 13]

Formalin test is a tonic pain model that is widely often used in the assay of antinociceptive activity.[14] The test was very effective model in ascertaining the analgesic activity of the plant extract. The early phase of the formalin test represents the transmission of nociceptive impulses while the second phase of the formalin test represents the events of central.[15] Centrally acting analgesics have effects on both phases whereas peripherally acting analgesics affects only the first phase.[16, 17] This is because the injection of formalin resulted in the release of various neurotransmitters including glutamate and aspartate in the dorsal horn.[18] This study has shown that *Vernonia hymenolepis* extract has activities that inhibit pain in both phases just like acetylsalicylic acid. Hence, the extract could be acting as a central analgesic like acetylsalicylic acid.

Acetylsalicylic acid is an analgesic that relieves aches and it is metabolized to integral part of human and animal metabolism.[19] It inhibits cyclooxygenase enzyme irreversibly unlike other NSAIDs since they affect more of COX-1 variant than the COX-2 variant of the enzyme.[20] They also have ability to suppress the production of prostaglandins and thromboxanes due to its irreversible inactivation of the cyclooxygenase enzyme required for prostaglandin and thromboxane synthesis.

Central analgesic drugs like narcotics inhibits both phases, while peripherally acting drugs like steroids (hydrocortisone and dexamethasone) and NSAIDs (indomethacine) acts mainly in the late phase (Trongsakul et al., 2003).

Conclusion

These studies have indicated that the use of *Vernonia hymenolepis* leaves by various communities to relief pain in toothache is justified since the extract exhibited significant antinociceptive activity compared to that of acetylsalicylic acid in formalin test. The antinociceptive activities were independent of sensorimotor retardation as demonstrated using pull-up test. No mice died at the dose administered (100mg/kg).

However, there is need for further bioguided assay on the aqueous leave extract in order to elucidate the active compound that would form the lead of the extract compound in developing new antinociceptive drugs. There is also need to assess the toxicological profile of the extract.

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