

Analgesic Potential of Acetone Leaf Extract of *Caesalpinia volkensii* Harms in Mice

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Abstract

Pain is defined as unpleasant feeling essential for body's defense system. It acts as a warning signal against disturbances in the body. Conventional antinociceptives are expensive and have many side effects. Continued use of these drugs may lead to tolerance and resistance. Medicinal plants have been used to relieve pain and form a better alternative. Herbal antinociceptives are affordable and have arguably fewer side effects. *Caesalpinia volkensii* (Harms) has pharmacological activities that include antimicrobial, immune modulatory properties and antimalarial. It is used locally by people in Embu County as analgesics. This study was designed to bioscreen the acetone leaf extracts of *C. volkensii* (Harms) for antinociceptive effects in mice. The plant parts were collected from Mbeere north sub-county, Embu County, Kenya. The samples were prepared and extraction of the active compounds carried out using acetone. Swiss albino mice were divided into five groups of five mice each: Normal, negative, reference and experimental group. Pain was induced experimentally using formalin and acetic acid. The experimental groups were treated with 50 and 100 mg/kg dose ranges of the plant extract. Mice were injected intraperitoneally with doses of the herbs, diclofenac and the vehicle. Thirty minutes later the animals were injected with 0.01ml of 2.5 % formalin in the sub planter region of the left hind paw and the other set with 0.4ml of 5% acetic acid. The total time spent lifting; biting, licking the paw and writhing were counted and scored. The acetone leaves extracts tested at different dose levels lowered paw licking time in a dose dependant manner. Further, the phytochemical screening results showed that the acetone leaves extracts of *C. volkensii* (Harms) have phytochemicals associated with antinociceptive activities. The study has established that the acetone leaves extract of *C. volkensii* (Harms) is effective in management of pain.

Keywords: *Caesalpinia volkensii* (Harms); writhing; Antinociceptive; Formalin; Acetic acid

Introduction

Pain is an unpleasant sensory affliction and emotional experience usually associated with actual or potential tissue damage, or described in terms of such damage [1]. It is not any disease, but it is manifested in certain disease or pathological conditions in the organism body. It acts as warning signal against disturbances in the internal or in the external environment of an individual which is essential for the organism's survival and wellbeing [2]. Inadequate pain relief is a known problem worldwide. Surveys show that many patients still suffer from moderate to severe pain [3], despite an increased focus on pain and the development of new standards for pain management [4]. The management of pain is a daily challenge in modern medicine despite the currently available wide range of analgesics. Conventional analgesics are expensive, have arguably many side effects such as gastric disorders, kidney, liver and heart failure, prolonged bleeding after injury and diabetes and continued use may lead to addiction and drug resistance. Almost all pharmacological treatments may produce side effects [5]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects against pain [6,7]. Traditional African herbal medicine (TAHM) is among the most ancient natural therapies and perhaps the oldest folk medicine currently practiced [8,9]. Alternative medicines are thought to possess many safe and effective phytochemicals useful in treating various disorders including pain [10].

Materials and Methods

Collection and preparation of plant materials

Fresh leaves of *C. volkensii* (Harms) were collected from their natural habitats on the basis of ethnobotanical information with

the help of local healers from Siakago division, Mbeere North Sub County in Embu County, Kenya. The samples were cleaned to ensure they are free of dust and other contaminants. They were transported to the department of Biochemistry and Biotechnology of Kenyatta University for studies. The samples were taxonomically identified and authenticated by an acknowledged taxonomist and a voucher specimen deposited at the Kenyatta University herbarium for future reference. The leaves were air dried at room temperature until completely dry. The dried leaves were then crushed by use of an electric mill to obtain fine powder which was stored at room temperature in air tight containers until used in extraction.

Extraction

200gms of the powder of the plant was weighed and put into a conical flask and labeled. 750 ml of acetone were added into the conical flask and corked. The mixture was allowed to stand overnight. The following day, the extract was filtered using Whatman No.1 filter papers. 250 ml of acetone was added to the remnant of each and allowed to stand for four hours. Second filtering was then done. The procedure was repeated until the solvent remained clear. The extracts were then

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concentrated using rotary evaporator at 56°C. The extracts were then placed in open beakers to allow any remaining acetone to evaporate until a sticky solid was obtained. This was stored at room temperature until use in bioassay.

Experimental animals

Swiss albino mice were used in this study. The mice breeding colony was acquired and bred in the animal breeding and experimentation facility of the department of Biochemistry and Biotechnology, of Kenyatta University. The animals were kept in standard cages and maintained under the standard laboratory conditions at room temperature and with 12 hours dark and 12 hours light cycle. They were fed on rodent pellets diet and supplied with water ad libitum. The ethical guidelines and procedures for handling animals were followed in the study.

Experimental design

Formalin-induced antinociceptive assay: The formalin induced antinociceptive assay was carried out as described by [11] Swiss albino male and female mice weighing between 20-25gms were divided into five groups of 5 animals each. Group I was intraperitoneally administered with 0.1ml normal saline (negative control). Group II was administered with 0.01ml of 2.5% formalin which was injected in the left hind paw (positive control). Group III was split into two groups of five animals each. Each group received treatment as follows; Group IIIa was intraperitoneally administered with 50mg/kg body weight dose of each plant extract, group IIIb was intraperitoneally administered with 100mg/kg body weight dose of each plant extract. Group IV was intraperitoneally administered with the standard drug, diclofenac (the reference drug) at a dose of 15 mg/kg body weight. Thirty after each dose administration, pain was induced by injecting 0.01 ml of 2.5% formalin in the left hind paw. The mice were placed in the prexi- glass box for observation. The time that the mice spent lifting, licking or biting the injected paw was recorded according to the pattern described by [12]. The two distinct periods of intensive licking, biting or lifting activity were identified and scored separately. The first period (early phase) was recorded between 0-5 minutes and the second period (late phase) was recorded between 15-30 minutes.

The percentage inhibition of the licking was calculated using the following formula

$$\frac{C - T}{C} \times 100$$

C

Where,

C = the vehicle control group value for the each phase,

T = the treated group value for the each phase.

Acetic-acid induced antinociceptive assay: This was carried out as described by [13]. Swiss male and female mice weighing between 20-25 grams were divided into five groups of 5 animals each. The groups will receive treatment as follows: Group I was intraperitoneally administered with administered with 0.1 ml of the vehicle, (normal saline). Group II was intraperitoneally administered with 0.4ml of 5% acetic acid solution. Group III was divided into two subgroups of five animals each. Each subgroup received treatment as follows; Group IIIa was intraperitoneally administered with 50mg/kg body weight of each plant extract. Group IIIb was intraperitoneally administered with 100mg/kg body weight of each plant extract. Group IV was intraperitoneally administered with standard drug, diclofenac

(the reference drug) at a dose of 15 mg/kg body weight. Thirty minutes after administration of the each treatments pain was induced by injection of 20 ml/kg 5% acetic acid in the right side of the belly intraperitoneally. The mice under experiments were then placed in prexi-glass box for observation. The number of abdominal constrictions (writhes) was counted 5 to 15 minutes after acetic acid injection. The percentage inhibition of the writhing was calculated using the following formula described in the previous section.

Qualitative phytochemical screening: Freshly prepared acetone leaf extracts of *C. volkensii* was subjected to qualitative phytochemical screening to identify presence or absence of major secondary metabolites using the protocol described by [14,15]. The classes of secondary metabolites, screened included alkaloids, saponins, terpenoids, sterols, flavonoids, cardiac glycosides and phenolics.

Data management and statistical analysis: The data on latency of pain response was obtained for all the animals in different groups, recorded and tabulated on a broad sheet using MS Excel. The results were expressed as mean \pm standard error of mean (SEM). Statistical significance of differences among group were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post *hoc* analysis to separate the means and obtain specific significant differences. $P \leq 0.05$ was considered significant. Analysis was done using Minitab statistical software.

Results

Antinociceptive activities of acetone leaf extracts of *C. volkensii* (Harms) on formalin induced pain in mice

Treatment of mice with acetone leaf extracts of *C. volkensii* at the dose levels of 50 and 100 mg/kg body weight reduced paw licking in the early phase by 14.59 % and 14.73% respectively (Table 1). The antinociceptive effectiveness of the two extract dose levels was not significantly different from each other as well as the controls ($p > 0.05$; Table 1; Figure 1). On the other hand, in the late phase, administration of acetone leaf extracts of *C. volkensii* at the dose levels of 50 and 100mg/kg body weight successfully reduced the formalin-induced pain in mice by 72.74% and 99.38 % respectively (Table 1; Figure 1). The antinociceptive effectiveness of the two extracts dose levels was not significantly different from each other and was comparable to the Diclofenac (reference drug) which reduced the formalin induced pain by 98.02% .This shows that the plant extracts was as effective as the reference drug ($p > 0.05$; Table 1; Figure 1).

Antinociceptive effects of acetone leaves extracts of *C. volkensii* (Harms) on acetic acid induced pain in mice

The treatment of mice with acetone leaf extracts of *C. volkensii* at dose levels of 50 mg/kg body weight reduced the number of writhes by 81.40%, which was comparable to the positive control (83.72 %) ($p > 0.05$; Table 2). However, the administration of 100 mg/kg body weight acetone leaf extracts of *C. volkensii* did not reduce the number of writhings (-80.23 %) highly concentrated extract molecules took longer to be absorbed across the membranes via filtration so that lower concentration of the extract in the 50 mg/kg body weight dose level was absorbed faster than 100 mg/kg body weight and caused antinociceptive activity in mice (Table 2; Figure 2).

Phytochemical screening

The acetone leaf extracts of *C. volkensii* tested positive for flavonoids, steroids and phenolics while alkaloids saponins, cardiac glycosides and terpenoids were absent (Table 3).

Animal Groups (N=5)	Treatment	Phase1	Phase2
Baseline	Formalin (2.5%)	258.60±10.9 ^a (2.78)	803.80±1.74 ^a (11.88)
Negative Control	DMSO (30%)	266.00±8.14 ^a (0.00)	718.40±2.66 ^a (0.00)
Positive Control	Diclofenac (15mg/kgbw)	259.40±3.44 ^a (2.63)	14.20±1.20 ^b (98.02)
Acetone Extract	50mg/kgbw	227.20±3.89 ^a (14.59)	195.80±2.60 ^b (72.74)
	100mg/kgbw	226.80±4.28 ^a (14.73)	4.00±0.71 ^{bc} (99.38)

Values are expressed as Mean ± SEM for five animals per group. Values with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test (p> 0.05). The figures in blankets represent % inhibition.

Table 1: Antinociceptive activities of acetone leaf extracts of *C. volkensii* on formalin induced pain in mice.

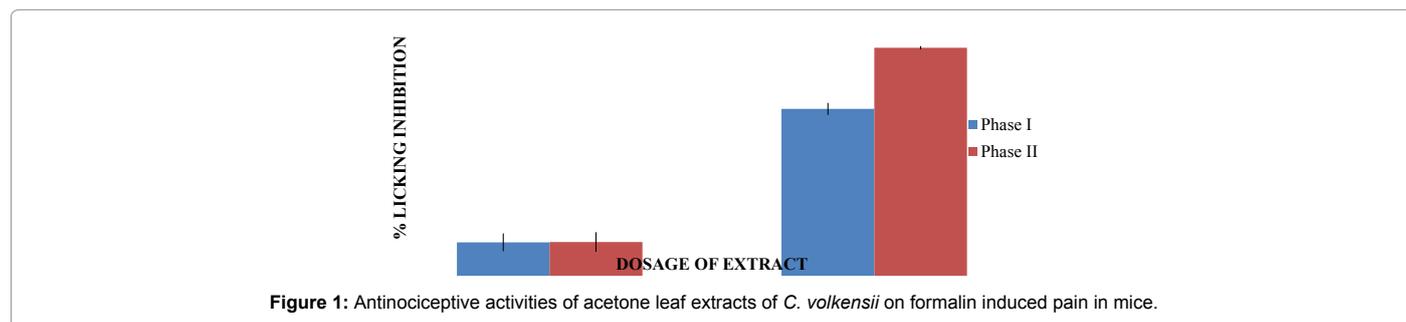


Figure 1: Antinociceptive activities of acetone leaf extracts of *C. volkensii* on formalin induced pain in mice.

Animal Groups (N=5)	Treatment	Number Of Writhings	% Inhibition
Baseline	Aceticacid (20ml/kgbw)	20.2±1.66 ^a	-17.44
Negative Control	DMSO (30%)	17.2±2.18 ^a	0.00
Positive Control	Diclofenac (15mg/kgbw)	2.80±0.97 ^b	83.72
Acetone Extract	50mg/kg bw	3.20±1.16 ^b	81.40
	100mg/kg bw	31.0±2.53 ^c	-80.23

Values are expressed as Mean ± SEM Values with the same superscript are not significantly different by ANOVA followed by Tukey's post hoc test (p>0.05)

Table 2: Antinociceptive properties of *C. volkensii* (Harms) in Acetic acid-induced pain in mice.

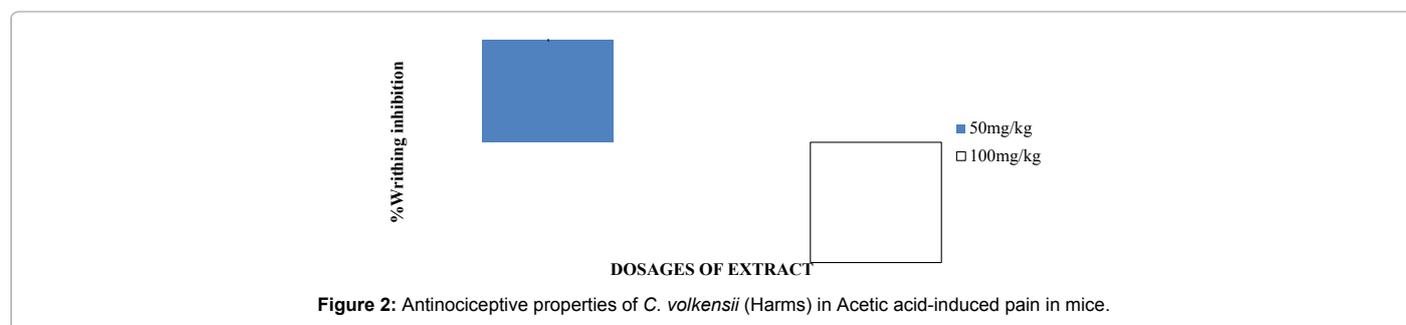


Figure 2: Antinociceptive properties of *C. volkensii* (Harms) in Acetic acid-induced pain in mice.

Discussion

In this study, the acetone leaf extract of *C. volkensii* (Harms) showed a significant antinociceptive effect by reducing formalin paw-licking time in both phases and acetic acid induced writhing, with a more potent activity in the second phase. This suggests both central and peripheral antinociceptive effects [16]. This central antinociceptive effect could have been caused possibly by inhibition of the nociceptive effects of serotonin, adrenaline, noradrenaline, prostaglandins, bradykinin, acetylcholine and adenosine and peripherally by inhibiting the release of endogenous mediators such as PGE2 prostaglandins E2 and PGE2 α in perionetal fluids as well as lipoxygenase which stimulates the nociceptive neurons [12].

The significant reduction in pain by the plant extracts in mice might be due to the presence of analgesic principles acting through the prostaglandin pathways. The mechanism of action of these two plant extracts can be postulated to be similar to that of non-steroidal

anti-inflammatory agents, such as ibuprofen, aspirin and diclofenac. The NSAIDs block the production of prostaglandins by truncating the COX1 pathway [17]. This blockage reduces sensitization of the peripheral nervous tissue resulting in less nerve stimulation and ultimately less pain [17].

The results of this study are similar to other previous studies on evaluation of antinociceptive activities of medicinal plant extracts [18,19]. That the acetone leaf extract of *C. volkensii* (Harms) showed significant antinociceptive effect by reducing formalin paw-licking and acetic acid writhings time in both phases is consistent with Gitahi and Hesseinzadeh, who worked on DCM methanolic leaf of *C. edulis* (Forssk) Vahl in laboratory animals and *M. officinalis* against pain respectively [18,19]. The dose ranges used in this study were within the dose ranges used in other (20-23,18,13) used dose ranges of 50, 100 and 150 mg/kg body weight while evaluating antinociceptive activity of DCM: methanolic leaf and root bark extracts of *C. edulis* (Forssk.) Vahl in laboratory animals [18].

Phytochemicals	<i>C. volkensii</i>
Alkaloids	-
Flavonoids	+
Steroids	+
Saponins	-
Cardiac glycosides	-
Phenolics	+
Terpenoids	-

Present phytochemicals are denoted by (+) sign, absent phytochemicals are denoted by (-) sign.

Table 3: Phytochemical composition of acetone leaf extracts *Caesalpinia volkensii* (Harms).

In this study high concentration (100mg/kg body weight) of the acetone leaf extracts of *C.volkensii* failed to reduce the writhes. This possibly may be explained by the lower dissociation in higher concentration which may have impeded filtration across the mucosal lining [24] (Figure 2).

The antinociceptive effect of the acetone leaf extract of *C.volkensii* (Harms) can be attributed to one or more groups of the phytoconstituents detected in the extracts. The flavonoids too from *M. officinalis* have been associated with anti-nociceptive effects. Flavonoids have been shown to cause anti-nociceptive effects by widely targeting prostaglandins [25] which are involved in the pain perception through moderating opioidergic mechanism [26]. The anti-nociceptive effects of *M. officinalis* are attributed to the flavonoids present in the extracts. On the other hand, studies have shown that flavonoids widely target prostaglandins which are involved in the pain perception through moderating opioidergic mechanism [26]. Alkaloids present in *K. macrophylla* showed analgesic actions [27,28] while aqueous extracts of *Radix Aconiti Carmichaeli* exhibited antinociceptive effect probably due to the presence of high content of mesaconitine alkaloids [29]. Among other actions, naturally occurring terpenoids present antinociceptive properties by inhibiting platelet aggregation, and interfering at the intracellular level, with several steps of signal transduction mechanisms [28].

Conclusion

In conclusion, the present study has demonstrated antinociceptive potential of acetone leaves extracts of *C. volkensii* (Harms) in mice models. The significant reduction in formalin induced pain when treated with standard drugs as well as different doses of extracts reflects that *C.volkensii* (Harms) acetone leaves extracts at 100mg/kg body weight were almost similar to the standard drug diclofenac sodium. For acetic acid induced pain, there was significant reduction of pain when treated with the standard drug diclofenac as well as *C.volkensii* (Harms) at doses 100 mg/kgbw and 50 mg/kgbw respectively. The acetone leaves extracts of *C.volkensii* contain different phytochemicals (secondary metabolites) which are responsible for antinociceptive activity. The acetone leaves extracts of *C.volkensii* (Harms) demonstrated antinociceptive properties and acts possibly centrally and peripherally.

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