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Anti-Nociceptive Activity of Fractionated Root Extract of *Strophanthus hispidus* DC (Apocynaceae)

Agbaje Esther Oluwatoyin, Fageyinbo Muyiwa Samuel*

Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, P.M.B. 12003, Idi-Araba-Lagos Nigeria

Abstract

Strophanthus hispidus DC belongs to the family of plants known as Apocynaceae, they are popularly known as poison arrow vine, brown *strophanthus* and hairy *strophanthus* in western part of Africa including Nigeria. This study investigated the anti-nociceptive property of ethyl acetate, n-butanol and aqueous fractions of the root extract of *Strophanthus hispidus* DC (Apocynaceae) in rodents of both sexes. The fractions, at a dose of 200 mg/kg each were given via oral route to the animals used in the various models- acetic acid-induced mouse writhing test, formalin- induced pain, Haffner's tail clip test, hot plate-induced pain and tail immersion test. In each of the models, the ethyl acetate fraction, n-butanol fraction and aqueous fraction of *Strophanthus hispidus* each possesses a significant ($P<0.05$, $P< 0.01$, $P< 0.001$) anti-nociceptive effect. The anti-nociceptive activity of the fractions was mediated through central and peripheral mechanism. The effect of the extract was comparable to that produced by peripheral analgesics the NSAIDs (aspirin) and centrally acting analgesic opioids (morphine) used as positive control in the various models employed. Phytochemical analysis of the fractions indicated the presence of flavonoids, cardiac glycosides, tannins, alkaloids and anthraquinones which also contributed to the anti-nociceptive activity of the extract.

These findings showed the plant as a novel therapy for pain.

Keywords: Anti-nociceptive, apocynaceae, morphine, Opioids, rodent, *Strophanthus hispidus*

1. Introduction

The use of plants as medicines predates written human history. Ethnobotany (the study of traditional human uses of plants) is recognized as an effective way to discover future medicines. In 2001, 122 compounds used in modern medicine were derived from "ethnomedical" plant sources; 80% of these have had an ethnomedical use identical or related to the current use of the active elements of the plant [12]. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including aspirin, digitalis, quinine, and opium [12]. The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The World Health Organization [30] estimates that 80 percent of the populations of some Asian and African countries presently use herbal medicine for some aspect of primary health care [12].

According to the WHO [30], more than 70% of the world's population must use traditional medicine to satisfy their principal health needs. In developing countries 80% population are using traditional medicine in primary medical problems [5, 13]. Plant drugs and herbal formulations are frequently

**Author for correspondence*
Email: samuelfageyinbo@yahoo.com

considered to be less toxic and free from side effects than synthetic ones [5]. Nature has offered a complete store-house of remedies for all ailments of mankind by providing drugs from herbs, whole plants and algae [2, 15, 23] most of which are of moderate toxicity relative to western medicines.

Strophanthus hispidus DC belongs to the family of plants known as Apocynaceae, they are popularly known as poison arrow vine, brown strophanthus and hairy strophanthus in western part of Africa including Nigeria [6]. A deciduous shrub of 5 m tall and up to 100 cm wide, having its stem bark dark grey in colour, with few lenticels, has been reported to have diverse medicinal uses; for example, in the Savannah Zone of West Africa, the latex and seeds of *Strophanthus hispidus* are used as arrow poison, while decoctions of root, stem bark or leaf are used externally to treat skin diseases, leprosy, ulcers, malaria, dysentery, gonorrhoea [8] and inflammatory [2]. In Nigeria and Ghana, the root decoction is ingested to treat rheumatic diseases, while in Togo; the root bark macerate is employed for treating oedema.

The objectives of this research is to evaluate the anti-nociceptive effect of aqueous, ethyl acetate and n-butanol fractions of the root extract of *Strophanthus hispidus* in mice, using standard in-vivo anti-nociceptive models and comparing its activity with modern day synthetic centrally and peripherally-acting analgesic agents.

2. Materials and Methods

2.1 Animals

Healthy Albino mice, 15–21 weeks old (18–30 g) of both sexes obtained from National Agency for Food and Drugs Administration and Control, Yaba (NAFDAC), were used in this study and maintained under standard laboratory conditions, as approved by the United States National Institute of Health (NIH) guide for Care and Use of laboratory animals and recommendation of IASP [32]. The animals, acclimatized for one week were fed on rodent diet (Livestock Feeds PLC, Ibadan, Oyo-State, Nigeria) and had free access to drinking water. However, they were fasted for at least 12 h prior to experimentation and all experiments were conducted between 9.00 am and 6.00 pm.

2.2 Preparation of the Fractions Of *Strophanthus hispidus*

400 ml of distilled water was added to 26.8 g of aqueous extract of *Strophanthus hispidus* in the beaker and dissolved properly. The resulting mixture was poured into a separating funnel clamped on the retort stand. To this mixture, different organic solvent was added in turns according to their polarity from non-polar to polar solvent: petroleum ether, ethyl acetate and n-butanol. The mixture was shaken vigorously to allow proper mixing and this was allowed to stand for some minutes for proper separation before removing the solvent.

The fractions were concentrated with rotary evaporator and the remaining was dried in water bath regulated at 40°C. The petroleum ether solvent did not pick any molecule from the crude extract of *Strophanthus hispidus*. The percentage yields of the resulting fractions were 2.6% n-butanol fraction, 1.62% ethyl acetate fraction and 83.81% aqueous fraction.

2.3 Pharmacological Studies

2.3.1 Mouse Writhing Test

Mice fasted overnight were divided into five groups of five animals each. The animals were treated as follows: Group 1: distilled water (10 ml/kg), Groups 2, 3 and 4 were treated with ethyl acetate, n-butanol and aqueous fractions of 200 mg/kg each respectively, Group 5: ASA (Acetylsalicylic acid) 100 mg/kg. All treatments were through oral administration. Sixty minutes after treatment, acetic acid (0.6% v/v in saline, 10 ml/kg i.p) was administered. The number of writhes (characterized by contraction of the abdominal musculature and extension of the hind limbs) were counted for 30 minutes

$$\text{Inhibition}\,(\%) = \frac{\text{Number of writhes (Control)} \bullet \text{Number of writhes (Treatment)}}{\text{Number of writhes (Control)}} \times 100$$

2.3.2 Formalin Test

Mice fasted overnight were divided into five groups of five animals each. The animals were treated as follows: Group 1: distilled water (10 ml/kg), Groups 2, 3 and 4 were treated with ethyl acetate, n-butanol and aqueous fractions of 200 mg/kg each respectively, Group 5: Morphine 10 mg/kg *s.c.* Sixty minutes after administration for the oral route and thirty minutes for

the subcutaneous route, formalin (20 µL of 1% solution) were injected into sub plantar tissue of the right hind paw of each mouse. The time (in seconds) spent in licking and biting responses of the injected paw, indicative of pain, were recorded for each animal. The responses of the mice were observed for 5 minutes (first phase) and 15-30 minutes (second phase) post formalin injection

$$\text{Inhibition}(\%) = \frac{\text{Reaction Time (Control)} \bullet \text{Reaction Time (Treatment)}}{\text{Reaction Time (Control)}} \times 100$$

2.3.3 Hot Plate Test

Mice that were used in this experiment were screened initially by placing the animals in turn on a hot plate set at 55 ± 1°C and animal which failed to lick the hind paw or jump (nociceptive responses) within 15s were discarded. Eligible animals were divided into five groups of five animals each. The animals were treated as follows: Group 1: distilled water (10 ml/kg), Groups 2, 3 and 4 were treated with ethyl acetate, n-butanol and aqueous fractions of 200 mg/kg each respectively, Group 5: Morphine 10 mg/kg *s.c.* sixty minutes after oral and thirty minutes after subcutaneous administration, the animal were placed on the hot plate and reaction time of animals were again recorded for two and half hours at thirty minutes interval. A post treatment cut-off time of 60 seconds was used.

$$\text{Inhibition}(\%) = \frac{(\text{Post - treatment Latency}) \bullet (\text{Pre - treatment Latency})}{(\text{Cut - off Time} \bullet \text{Pre - treatment Latency})} \times 100$$

2.3.4 Tail Immersion Test

Mice that were used in this experiment were screened initially by dipping the lower 5 cm portion of the tail into hot water bath maintained at 55°C ± 0.5 to induce pain and animal that failed to attempt to withdraw the tail in 10 seconds were discarded (Janssen *et al.*, 1963). Eligible mice were divided into five groups of five animals each. The post-treatment reaction time of all mice was determined after which the animals were treated as follows: Group 1: distilled water (10 ml/kg), Groups 2, 3 and 4 were treated with ethyl acetate, n-butanol and aqueous fractions of 200 mg/kg each respectively, Group 5: ASA 100 mg/kg *s.c.* The times in seconds to withdraw the tail clearly out of the water were taken as the reaction time. Reaction time was taken after oral administration of the extract at 30, 60, 90, 120, and 150 minutes. A post treatment cut-off time of 30 s was use.

$$\text{Inhibition}(\%) = \frac{(\text{Post - treatment Latency}) \bullet (\text{Pre - treatment Latency})}{(\text{Cut - off Time} \bullet \text{Pre - treatment Latency})} \times 100$$

2.3.5 Haffner's Tail Clip Test

Mice that were used in this experiment were screened initially by applying a metal artery clip to the root of the tail to induce pain and animal that failed to attempt to dislodge the clip in 10 seconds were discarded. Eligible mice were divided into five groups of five animals each. The pre-treatment reaction time of all mice to clip were determined after which the animals were treated as follows: Group 1: distilled water (10 ml/kg), Groups 2, 3 and 4 were treated with ethyl acetate, n-butanol and aqueous fractions of 200 mg/kg each respectively, Group 5: Morphine 10 mg/kg *s.c.* Reaction time of each mouse was determined 60 mins post treatment for oral administration and 30 mins post treatment for subcutaneous administration (Adeyemi*et al.*, 2004). A post-treatment cut-off time of 30 s was used.

$$\%\,\text{Inhibition} = \frac{(\text{Post - treatment Latency}) \bullet (\text{Pre - treatment Latency})}{(\text{Cut - off Time} \bullet \text{Pre - treatment Latency})} \times 100$$

2.4 Statistical Analysis

Results obtained were expressed as Mean ± SEM. The data were analyzed using one way ANOVA followed by Tukey's multiple comparison test or by two- way ANOVA followed by Bonferroni posttest using GraphPad Prism 5 (GraphPad Software Inc., CA, USA). Results were considered significant when $p<0.05$, $p<0.01$ and $p<0.01$.

3. Results

3.1 Acetic Acid Induced Writhes

Intraperitoneal injection of acetic acid elicited the writhing syndrome in control mice with 121 ± 11.18 writhes counted in 30 minutes. Ethyl acetate fraction, n-butanol fraction and aqueous fraction of the extract statistically ($P < 0.001$, $P < 0.001$) inhibit the pain produced by the acetic acid with 77.36% (27.4 ±6.12), 37.36%, (75.8 ±9.15) and 71.07% (34 ±2.78) inhibition respectively at a dose of 200 mg/kg each. The highest

inhibition was observed with ethyl acetate fraction which was higher than that produced by acetic salicylic acid (Aspirin) (74.05%) (31.4 ± 2.42). Aqueous fraction produced an inhibition (71.07%) that effectively compared with aspirin while the lowest inhibition was observed with the n-butanol fraction (37.36%). (Fig. 1).

3.2 Formalin-Induced Pain

In the first phase, injection of formalin into the sub-plantar tissue of the right hind paw of control mice produced nociceptive response of biting and licking of the paw with a duration of 105.54 ± 4.03 seconds. Ethyl acetate fraction, n-butanol fraction and aqueous fraction of the extract statistically ($P < 0.01$, $P < 0.001$) inhibited the pain with 45.15 %, 61.15 % and 61.91 % respectively at a dose of 200 mg/kg each. This effect was less than that produced by morphine (98.67 %). In the second phase, ethyl acetate fraction, n-butanol fraction and aqueous fraction of the extract statistically ($P < 0.01$, $P < 0.001$) inhibit the pain induced by formalin with 77.03 %, 77.42 % and 79.93 % respectively. This effect was greater than that produced in the first phase but still less than that produced by morphine (Fig. 2).

3.3 Haffner's Tail Clip Induced Pain Test

Application of the metal artery clip unto the tail of animals in the control group elicited reactions towards clip removal with the post-treatment latency being 1.94 ± 0.31s, 1.89 ± 0.34s, 1.79 ± 0.33s and 1.29±0.16s measured at 60 minutes, 90 minutes, 120 minutes and 150 minutes respectively with a pre-treatment latency of 2.08 ± 0.58s. Ethyl acetate fraction, n-butanol

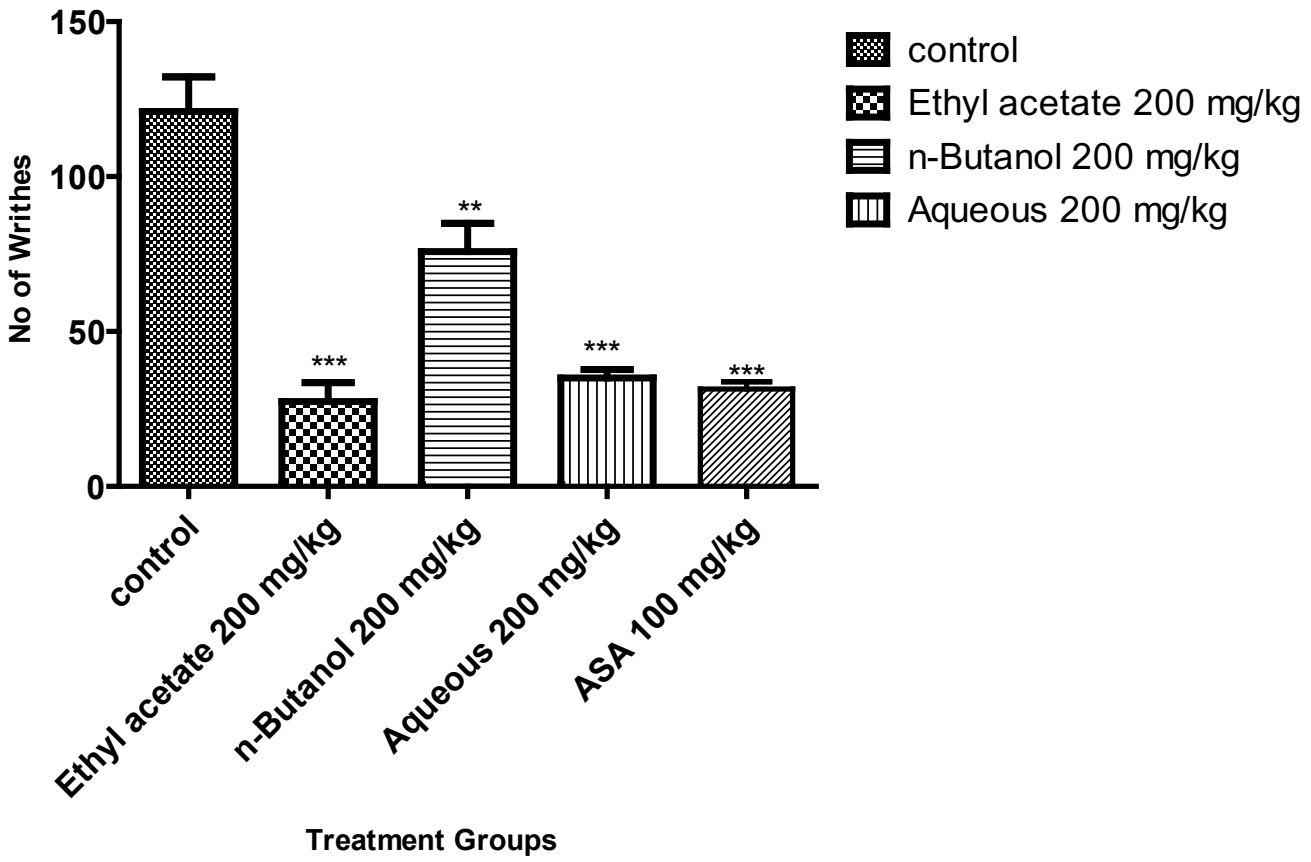

Fig. 1. Effect of ethyl acetate fraction, n-butanol fraction and aqueous fraction extracts of *Strophanthus hispidus* on acetic acid induced writhes test.
ASA: Acetylsalicylic acid (Aspirin)
$^{***}P < 0.001$ statistically significant compared to control, $^{**}P < 0.01$ statistically significant compared to control (one way ANOVA followed by Tukey's multiple comparison test).

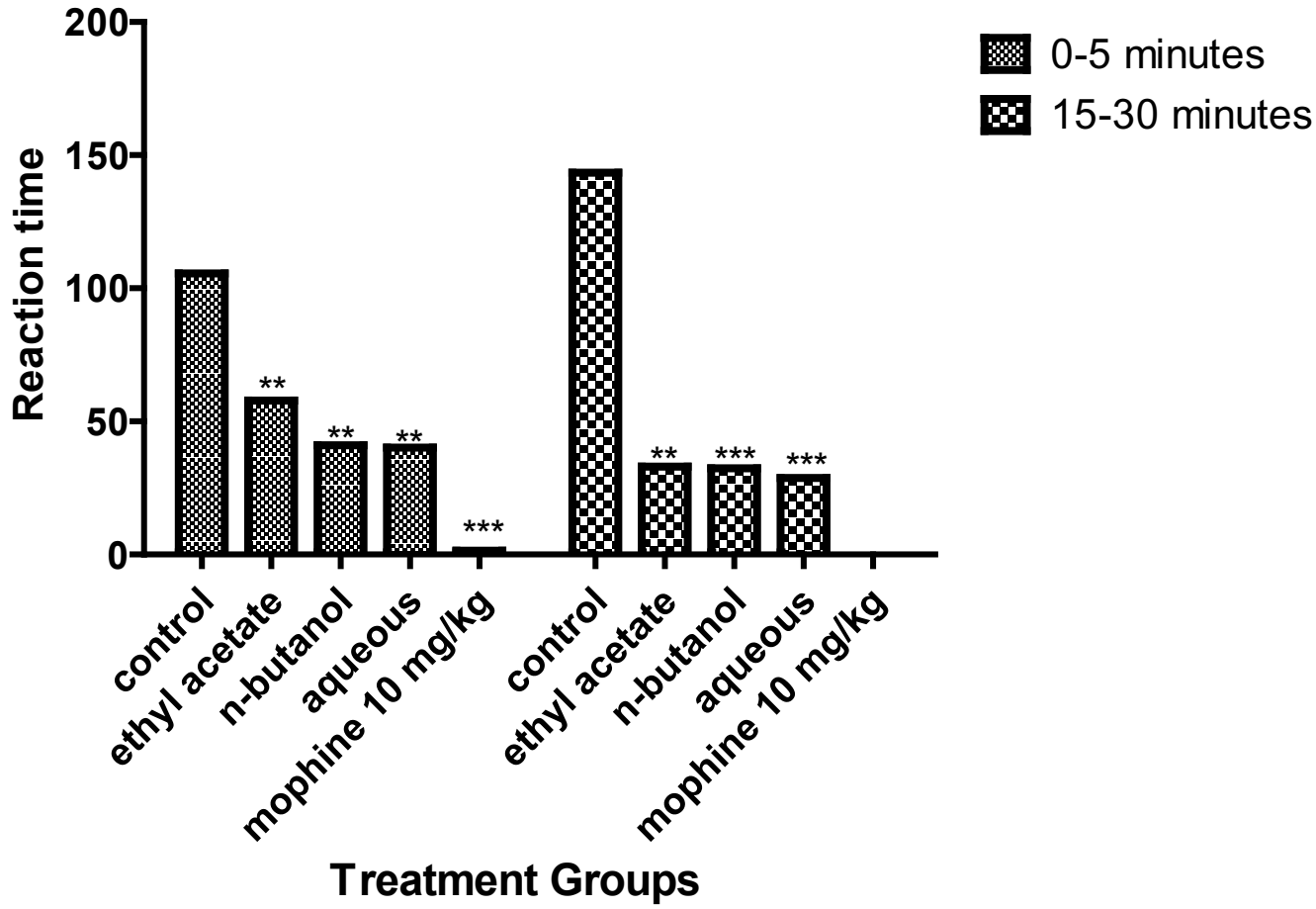

Fig. 2. Effect of ethyl acetate fraction, n-butanol fraction and aqueous fraction extracts of *Strophanthus hispidus* on formalin induced pain in mice.
$^{**}P< 0.01$ statistically significant compared to control, $^{***}P< 0.001$ statistically significant compared to control (one way ANOVA followed by Tukey's multiple comparison test).

fraction and aqueous fraction of the extract statistically ($P < 0.05$, $P < 0.001$) with 31.31 %, 65.11 % and 65.21 % respectively at 120 minutes post-treatment at 200 mg/kg each. Morphine 10 mg/kg produced a peak inhibition (100%) at 90 minutes post treatment. The effect produced by n-butanol fraction and that of aqueous fraction were similar and greater than that produced by ethyl acetate fraction but less than that produced by morphine (Table 1).

3.4 Tail Immersion-Induced Pain

Ethyl acetate fraction, n-butanol fraction and aqueous fraction of the extract of *Strophanthus hispidus* statistically ($P < 0.05$, $P <0.01$, $P < 0.001$) inhibit pain induced by tail immersion with highest inhibition of 26.85%, 51.28% and 54.72% respectively produced at 90 minutes post-treatment at a dose of 200 mg/kg each. The effect produced by n-butanol fraction and aqueous fraction was comparable to that produced by 10 mg/kg morphine (67.89% inhibition) at 120 minutes (Table 2).

3.5 Hot Plate-Induced Pain

Ethyl acetate fraction, n-butanol fraction and aqueous fraction of the extract of *Strophanthus hispidus* statistically ($P < 0.05$, $P <0.01$, $P < 0.001$) inhibit pain induced by placing the mice on hot plate maintained at 55°C ± 1with highest inhibition of 25.28%, 50.61% and 51.91% respectively produced at 90 minutes post-treatment at a dose of 200 mg/kg each. Morphine 10 mg/kg produced highest inhibition (100%) at 60 minutes. The effect produced by n-butanol fraction and aqueous fraction were more than that of ethyl acetate fraction but less than that produced by morphine (Table 3).

Table 1: Effect of ethyl acetate fraction, n-butanol fraction and aqueous fraction extracts of *Strophanthus hispidus* on tail clip induced pain in mice

		Reaction time (secs)				
		Pre treatment	Post treatments			
Treatments	Dose (mg/kg)		60 minutes	90 minutes	120 minutes	150 minutes
Control (DW)	10 ml/kg	2.08 ± 0.58	1.94 ± 0.31	1.89 ± 0.34	1.79 ± 0.33	1.29 ± 0.16
Ethyl acetate fraction	200	3.34 ± 0.15	13.46 ± 1.23*	17.25 ± 1.86*	21.08 ± 1.49[a]	17.00 ± 1.14
		Inhibition%	17.83	24.55	31.31	24.11
n-Butanolfraction	200	1.96 ± 0.20	9.31 ± 1.47	29.75 ± 4.60[a]	39.75±7.86[a]	25.25 ± 6.51*
		Inhibition%	12.66	47.88	65.11	40.13
Aqueous Fraction	200	1.95 ± 0.11	10.91 ± 1.54	31.24 ± 4.68[a]	39.80 ± 7.97[a]	28.26 ± 6.47[a]
		Inhibition%	15.44	50.46	65.21	45.32
Morphine	10	2.21 ± 0.44	57.08 ± 1.64[a]	60.00 ± 0.00[a]	56.8 ± 1.33[a]	53.12 ± 1.32[a]
		Inhibition%	94.95	100	94.96	88.10

DW: Distilled Water

Mean ±S.E M: Standard Error of Mean

*P< 0.05 statistically significant compared to control, [a]P< 0.001 statistically significant compared to control (2Way ANOVA followed by Bonferroni posttests).

Table 2: Effect of ethyl acetate fraction, n-butanol fraction and aqueous fraction extracts of *strophanthus hispidus* on tail immersion-induced pain in mice

		Reaction time (secs)				
		Pre treatment	Post treatments			
Treatments	Dose (mg/kg)		60 minutes	90 minutes	120 minutes	150 minutes
Control (DW)	10 ml/kg	1.31 ± 0.14	1.45 ± 0.13	1.48 ± 0.09	1.42 ± 0.17	1.23 ± 0.09
Ethyl acetate fraction	200	2.38 ± 0.38	13.28 ± 0.79	17.85 ± 1.20*	11.05 ± 0.78	7.96 ± 0.55
		Inhibition %	18.92	26.85	15.05	9.68
n-Butanolfraction	200	1.94 ± 0.22	15.11 ± 1.23[a]	31.71 ± 5.03[b]	22.71 ± 0.90[a]	16.64 ± 0.73[a]
		Inhibition %	22.68	51.28	35.77	25.32
Aqueous Fraction	200	1.92 ± 0.36	17.86 ± 1.60[a]	33.70 ± 5.23[b]	24.12 ± 1.53[b]	18.45 ± 0.73[b]
		Inhibition %	27.45	54.72	38.22	28.46
Morphine	10	3.66 ± 0.45	37.92 ± 2.29[b]	41.67 ± 2.31[b]	41.91 ± 2.26[b]	36.61 ± 2.17[b]
		Inhibition %	60.8	67.46	67.89	58.48

DW: Distilled Water

±S.E M: Standard Error of Mean

*P< 0.05 statistically significant compared to control, [a]P< 0.01 statistically significant compared to control, [b]P< 0.001 statistically significant compared to control (2Way ANOVA followed by Bonferroni posttests).

Table 3: Effect of ethyl acetate fraction, n-butanol fraction and aqueous fraction extracts of *strophanthus hispidus* on hot plate induced pain in mice

		Reaction time (secs)				
		Pre treatment	Post treatments			
Treatments	Dose (mg/kg)		60 minutes	90 minutes	120 minutes	150 minutes
Control (DW)	10 ml/kg	2.52 ± 0.01	2.46 ± 0.08	2.44 ± 0.08	2.30±0.05	2.17±0.04
Ethyl acetate fraction	200	2.79 ± 0.27	12.88 ± 1.07*	17.25 ± 1.58*	12.65±0.87	8.56±0.76
		Inhibition%	17.64	25.28	17.24	10.09
n-Butanolfraction	200	3.56 ± 0.44	15.24 ± 1.69b	32.16 ± 5.39b	22.69±0.91a	15.91±1.67a
		Inhibition%	20.70	50.67	33.89	21.88
Aqueous Fraction	200	2.97 ± 0.13	17.33 ± 0.81b	32.57 ± 4.68a	21.50±1.70a	15.42±1.31a
		Inhibition%	25.18	51.91	32.50	15.42
Morphine	10	3.89 ± 0.43	60.00 ± 0.00b	55.96 ± 1.69b	52.49±1.22b	43.51±3.26b
		Inhibition%	100	92.80	86.62	70.61

DW: Distilled Water

±S.E M: Standard Error of Mean

*P< 0.05 statistically significant compared to control, aP< 0.01 statistically significant compared to control, bP< 0.001 statistically significant compared to control (2Way ANOVA followed by Bonferroni posttests).

Table 4: Results of the phytochemical analysis of the aqueous fraction, n-butanol fraction and ethyl acetate fraction of the root extract of *Strophanthus hispidus*

Constituents	Ethyl acetate	Aqueous	n-Butanol
Tannins	Absent	Present	Present
Alkaloids	Present	Absent	Absent
Anthraquinones	Present	Present	Present
Cardiac glycosides	Present	Present	Present
Flavonoids	Absent	Present	Present

4. Discussion

Pain is an unpleasant sensory emotional experience associated with actual or potential tissue damage, described as a subjective, unpleasant, physical and psychological experience observed as a result of stimulation of identifiable nerve fibres with defined pathways to the brain via the spinal cord [19]. Pain often results from tissue damage that stimulates nociceptive receptors (nociceptive pain) but pain may also occur without nociception; here it could be as a result of damage to neural structure (neuropathic pain or neuralgia).

While the former often acute, self-limiting after healing and responds easily to analgesics, the latter is very difficult to treat, there may or may not be evidence of injury, causing chronic pain and will persist long after the initial injury has healed [14, 27]. In this study, the mouse writhing, formalin, and tail clip, hot plate and tail immersion tests have been used to screen for analgesic activity of aqueous, n-butanol and ethyl acetate fractions of the root extract of *Strophanthus hispidus*.

The mouse writhing test is useful for the evaluation of mild analgesic non-steroidal anti-inflammatory compounds [3, 7, 18] and peripherally acting drugs like ASA have been reported to exhibit analgesic activity in the writhing test only [31]. Writhings induced by acetic acid involves stimulation of the peripheral receptor system and is partly attributed to the local peritoneal receptors found at the surface of the cells lining the peritoneal cavity [31]. Prolonged irritation of the peritoneal cavity by acetic acid results in increase in the levels of peritoneal fluid prostaglandins, like PGE_2 and

$PGF_2\alpha$, which enhances inflammatory pain by increasing capillary permeability [29, 31].

The analgesic effect demonstrated by ethyl acetate, n-butanol and aqueous fractions of the root extract of *S. hispidus* in the mouse writhing test in this study suggest a peripheral mechanism of action involving direct action on nociceptors, direct inhibition of prostaglandin action or indirect inhibition of prostaglandin synthesis by inhibition of Cyclo-oxygenase (COX) activity. The effectiveness of these fractions of *S. hispidus* extract in this test indicates its potential usefulness in the treatment of acute pain as the abdominal constriction test is said to be indicative of acute pain [10].

The formalin test produces distinct biphasic nociceptive response; the first transient phase (early phase) is caused by the direct effect of formalin on sensory C-fibers, and the second prolonged phase (late phase) is associated with the development of the injury induced spinal sensation, responsible for facilitated pain processing, a central sensitization of the dorsal horn neuron occurred during inflammation pain [4, 11, 24]. Injection of formalin causes immediate and intense increase in the spontaneous activity of afferent C fibers and evokes distinct quantifiable behavior indicative of pain [31] biting and licking of the injected paw as observed in this study. The early phase is due to direct effect of formalin on nociceptors while the late phase is a tonic response involving inflammatory processes and neurons in the dorsal horns of the spinal cord are activated [28, 31]. According to Cowan [10], the formalin test can also be used to indicate the potential of analgesic agents to treat chronic pain due to inflammation.

Results of the present study showed that the n-butanol and aqueous fractions of the root extract of *S. hispidus* inhibit both the early and the late phases of formalin-induced pain, while the ethyl acetate fraction showed a mild inhibition at the early phase thus suggesting its central and peripheral anti-nociceptive actions. However, the fractions of the root extract of *S. hispidus* produced greater inhibition at the late phase than the early phase and this effect was comparable to that produced by the standard drug (morphine).In agreement with literature, morphine was effective in both phases of the formalin test. Findings in the formalin test suggest that the ethyl acetate, aqueous and n-butanol fractions of the root extract of *S. hispidus* acts through peripheral mechanism of action as established in the mouse writhing test and also via central mechanism because drugs which act mainly centrally, like narcotic analgesics (e.g. morphine) inhibit both phases of the formalin test [1, 9, 25] as demonstrated by the fractions.

The involvement of central mechanisms in the analgesic activity of ethyl acetate, n-butanol and aqueous fractions of the root extract of *S. hispidus* was further evaluated using the tail clip and hot plate tests as centrally acting analgesic drugs like morphine elevate the pain threshold of rodents towards heat and pressure [26]. As reported by Ramabadran and Bansinath [22], increase in the pain reaction time (latency period) indicates the level of analgesia induced by the drug or extract. Ethyl acetate, n-butanol and aqueous fractions of the root extract of *S. hispidus* showed a significant ($P<0.05$) increase in pain threshold in the mice. In the tail clip model, the effect peaked at 120 minutes with ethyl acetate (31.31%) having a very mild effect compared to the n-butanol (65.11%) and aqueous (65.21%) fractions. This might be an indication of the involvement of both μ-opioid and κ-opioid receptors. Increase in stress tolerance capacity of the animals indicates the possible involvement of a higher center. The hot plate test involves the spinal reflex [20] and measures the complex response to a non-inflammatory, acute nociceptive input [31]. The effect of the fraction in the hot plate model peaked at 90 minutes post treatment with ethyl acetate (25.28%) also having a mild effect compared to n-butanol (50.67%) and aqueous (51.91%) fractions. As a result of these, involvement of central mechanism is further established.

Tail immersion test procedure consists of behavioral method that has been developed to study nociception in animals [22]. The animal response in this test is usually integrated at the lower level in the central nervous system, thus, giving information about the pain threshold. It is therefore, used to detect narcotic and non-narcotic analgesics. In this model, the effect peaked at 90 minutes with ethyl acetate (26.85%) having a very mild effect compared to the n-butanol (51.28%) and aqueous (54.72%) fractions.

Tannins are present in the aqueous and n-butanol fractions, anthraquinones and cardiac glycosides are present in three fractions (ethyl acetate, aqueous and n-butanol) while flavonoids are present in the aqueous and n-butanol fractions. One or a combination of this

phytoconstituents may be responsible for the analgesic activity observed with the ethyl acetate, n-butanol and aqueous fractions of the root extract of *S. hispidus* in this study. Alkaloids which generally have wide pharmacological actions are analgesics and narcotics [21] and their ability to cross the blood-brain barrier and interact with various neurotransmitter receptors determine much of their pharmacology [16]. Some alkaloids have also been reported to possess analgesic and anti-inflammatory activity [16]. Flavonoids have been shown to possess various pharmacological and biochemical actions including analgesic, antipyretic and anti-inflammatory properties [16]. Specific flavonoids and related compounds e.g. genistein, kaempferol, quercetin, resorcinol, and resveratrol have been reported to exhibit Cyclooxygenase (COX)-2 inhibition activity and dose-dependent decreases in inflammatory-mediating cytokine TNFα [17].

5. Conclusion

The results obtained in this study demonstrated that the ethyl acetate, n-butanol and aqueous fractions of the root extract of *Strophanthus hispidus* possess analgesic property mediated through peripheral and central mechanisms.

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