

***In-vivo* analgesic activity of ethanol leaf extract of *Sphenocentrum Jollyanum* in albino mice.**

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Abstract

This study assessed the *in-vivo* analgesic activity of ethanol leaf extract of *Sphenocentrum jollyanum* in albino mice using analgesic models such as acetic acid-induced abdominal writhing, formalin-induced paw licking and thermally-induced pain in mice. Acetylsalicylic acid (ASA) was used as the reference drugs for the acid-induced abdominal writhing and formalin-induced paw licking while Dexamethasone was used as standard drug for thermally-induced pain. A total of seventy-five matured Swiss Albino mice of both sexes weighing between 15 – 25 g (divided into three groups of 25 mice each for the 3 different studies) were used for this study. They were randomly divided into 5 groups of five mice each. Group I served as the normal control and received 10 mL/kg body weight (bw) distilled water. Group II (Standard control), received standard drugs- Acetylsalicylic acid (ASA) or Dexamethasone. Groups III, IV and V received 474.34, 948.68 and 1423.03 mg/kg bw ethanol leaf extract of *S. jollyanum* respectively. All administration was done intraperitoneally. The animals were fed normal rat pellet and had water *ad libitum*. The results showed a significant ($p < 0.05$) dose dependent reduction in the acetic acid-induced abdominal writhing and formalin-induced paw licking at all doses (Groups III -V) compared with acetylsalicylic acid standard drug Group II. In the acetic acid-induced writhing and formalin paw licking models, the extract had the highest writhing and paw licking effects of 37.40 ± 0.68 and 28.60 ± 0.60 at the highest dose of 1423.03 mg/kg respectively. Similarly, the extract caused a dose-dependent increase in percentage inhibition of abdominal writhing and paw licking effects at total timing of 105 mins with the highest dose of 1423.03 mg/kg respectively. Additionally, the extract (groups III-V) and standard drug (II) demonstrated a dose-dependent ($p < 0.05$) increase in PRT compared with control group I, with the standard drug dexamethasone (group II) having the highest effect. *Sphenocentrum Jollyanum* leaves extract also showed significant increase in PRT from 135 to 190% compared to control group I. The result obtained from this study suggests that the extract of *Sphenocentrum Jollyanum* leaf may be an effective analgesic agent that may act by the inhibition of prostaglandin synthesis or via peripheral mechanism of pain inhibition.

Keywords: *Sphenocentrum jollyanum*; pain; analgesic; abdominal writhing; paw licking.

1. Introduction

Pain is a warning mechanism that protects an organism by making it withdraw from harmful stimuli that is primarily associated with injury or may be a threat of injury. It is also a complex experience in which cognitive, affective and behavioural features, representing psychological conditions are affected [1]. The International Association for the Study of Pain (IASP) defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain can be acute or chronic and can develop into chronic pain syndrome, if left untreated [2]. Different reasons such as harmful heat, stretch, electrical flow, necrosis, inflammation, laceration and spasm can bring about pain [3]. A wide variety of degenerative diseases such as rheumatoid arthritis, polymyalgia rheumatica, as well as heart, asthma, cancer and inflammatory bowel diseases have been reported to be associated with inflammatory processes and pains [1]. Pain therefore, is an indication of several illnesses and it is predicted that about 80–100% of the population will experience pain at some point in their life [4]). Although pain is mainly considered as a defense mechanism which is created when tissue is damaged and caused a person to show reaction with removal of pain stimulant [5], about 22% of primary care patients suffer from pain which persists for more than six months and in some cases, the percentage rises to 50% and is related to significant impairment of social functioning and quality of life [6].

The global burden of morbidity due to pain has been reported to be huge and due to its frequent occurrences, it is a health problem that reduce the quality of life and possesses socioeconomic effect both on the patients and their families [7]. Treatment modalities for pains include physical therapies such as heat or cold packs, massage, hydrotherapy and exercise; psychological therapies such as cognitive behavioural therapy, relaxation techniques and meditation; mind and body techniques such as acupuncture; occupational therapy and pain-relieving medicines such as analgesics, antidepressant, anticonvulsant [8]. Two types of over-the-counter (OTC) pain medications; acetaminophen (Panadol) and nonsteroidal anti-inflammatory drug (NSAIDs) such as aspirin, naproxen and ibuprofen have also been used to treat pains [9]. Gastrointestinal irritation, bronchospasm, fluid retention, and extension of bleeding has been reported as some adverse effects associated with several over the counter anti-inflammatory and analgesic drugs [10].

Over the years, good health has been the greatest challenge to mankind globally. Long term pains impose undesirable mental effects. There is a report on several ways to decrease or eliminate pain [11]. Medicinal plants have been suggested to present natural, effective substances for prevention or treatment of pain-related conditions [12]. Natural products from plants, animals and minerals has also been reported to have contributed in the development of new drugs and for treating human diseases [13]. The use of plants for food and medicinal purposes in the management of various disease conditions has increased due to the unavailability of modern health facilities, relative availability of medicinal herbs, poverty and recent relations that they possess active compounds that may be responsible for different biological and pharmacological actions with no adverse effects [14]. One of such plant is *Sphenocentrum jollyanum* used commonly in south eastern part of Nigeria for the treatment of pains. It is an undergrowth shrub plant that thrives in deep shade and commonly found in the tropical forest of West Africa. *Sphenocentrum jollyanum* belongs to the family *Menispermaceae* and is locally called Oji-enyi, Ajo or Akerejupon

and Ibong Isong by the Igbos, Yorubas and the Ibibios respectively [15]. The leaves are wedge in shape and is about 5-12 cm wide and smooth on both sides and can grow up to about 20 cm long with a small arrowed apex [16]. The leaves have been reported to be rich in phytoconstituents such as alkaloids, saponins, tannins, phlobatannins, flavonoids and cardiac glycosides, with the LD₅₀ of 4743.42 mg/kg body weight [17]. Different parts of the plants such as the leaves, stem and roots have been reported to possess ethnomedicinal values and has effect in central nervous system (CNS) diseases such as psychiatric disorders, inflammation and pains [18]. It is also used in the treatment of blood pressure, breast tumor, irregular menstrual cycle, and diabetes mellitus [16;19;20;21;22]. *Sphenocentrum jollyanum* have also been found to be potent against polio type-2 viruses [23]. The plant extract is popular in chronic wound dressing, feverish conditions, cough and as an aphrodisiac [24,16,25]. Earlier studies have shown that the ethanolic extract of *Sphenocentrum jollyanum* root increased libido and enhanced sexual behaviour in male mice [26].

Studies have also shown that the metabolic stem bark extract of the plant possesses significant hepatoprotective and antioxidant properties against carbon tetrachloride-induced stress in rats [27]. Petroleum ether and methanol extracts of *S. Jollyanum* leaves has been reported in previous studies to possess significant in-vitro analgesic and antipyretic properties [28]. This study aims at assessing for analgesic activity of ethanolic leaf extract of *Sphenocentrum jollyanum* in-vivo in experimental mice.

2. Materials and Methods

2.1 Chemical and instrument

Acetylsalicylic acid (ASA) and dexamethasone were purchased from Sigma Aldrich (St. Louis, MO, USA) were used as the standard drugs. Acetic acid was obtained from Merck (Darmstadt, Germany) while formalin was obtained from Nihon Shiyaku Industries (Japan). Ethanol was obtained from JHD, China. Scientific hotplate (Thomas autoregulated temperature model, USA) and Ohaus weighing scale (Ohaus Corporation, USA, model: Scout pro-SPU 202) were from the Pharmacognosy Laboratory, University of Uyo, Uyo. All other chemicals and reagents used were of analytical grade and acquired from local firms

2.2 Sample Collection and Preparation of extract

Fresh leaves of *Sphenocentrum jollyanum* (*S. Jollyanum*) commonly were collected from Aka in Ibiono Ibom Local Government Area of Akwa Ibom State, Nigeria. They were identified and authenticated by a taxonomist in the Department of Pharmacognosy and Natural Medicine, University of Uyo, Akwa Ibom State with a voucher no NUUH:040/15 and the specimen deposited at the herbarium. The leaves were washed with clean water to remove accumulated dirt's and air-dried for two weeks at room temperature. Eight hundred and thirty-three point seven grammes (833.7 g) of the powdered sample was macerated in 1.5 litre of 60% ethanol for 72 hours and intermittently shaken to allow the solvent to solubilized the active ingredients. The mixture was filtered using muslin cloth and thereafter filtered with Whatman No. 1 filter paper to obtain a yellowish brown coloured filtrate. The filtrate was concentrated in a water bath at 45°C to obtain a paste-like gel extract that was further dried in a desiccator between 28 to 33 °C to completely eliminate all the solvent to yield 188.5 g of crude extract.

2.3 Experimental Animals and Design

Seventy-five matured Swiss Albino mice of both sexes weighing between 15 – 25 g were procured from the Animal House, Department of Pharmacognosy and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, were used for this study. The mice were kept in stainless steel cages under laboratory conditions, fed with commercial pelleted feed (Ladokun Feeds, Nigeria Ltd) and were allowed distilled water *ad libitum*. The animals were acclimatized for two weeks before the commencement of administration. The mice were randomly divided into 5 groups of five mice each. The care and use of the animals were in compliance with standard protocols of the National Institute of Health guidelines for the care and use of Laboratory Animals [29].

The treatment groups were as follows:

Group I: Normal control that received 10 mL/kg body weight distilled water.

Group II: Standard control that received standard drug; Acetylsalicylic acid (ASA) and Dexamethasone respectively

Group III: Received 474.34 mg/kg bw ethanol leaf extract of *S. jollyanum*

Group IV: Received 948.68 mg/kg bw ethanol leaf extract of *S. jollyanum*

Group V: Received 1423.03 mg/kg bw ethanol leaf extract of *S. jollyanum*

2.4 Determination of the Analgesic activity of *Sphenocentrum jollyanum*

The following methods were adopted in this study to assess for analgesic property of *Sphenocentrum jollyanum*;

2.4.1 Acetic acid-induced abdominal writhing

Analgesic activity of ethanol leaf extracts of *Sphenocentrum jollyanum* against acetic acid-induced writhing was performed according to the procedure by [30;31;32]. Twenty-five Swiss albino mice (15-22g) of both sexes were randomly divided into 5 groups of 5 mice each and fasted for 24 hours but still had water *ad libitum*. Control group I received 10 mL/kg distilled water, group II received 50 mg/kg acetylsalicylic acid (ASA) which serves as a standard drug while groups III, IV and V were given 474.34mg/kg, 948.68mg/kg and 1423.03mg/kg doses of ethanol leaf extract of *Sphenocentrum jollyanum* respectively. All administrations were done intraperitoneally.

After 30 minutes of administration of the extract and the standard drug, 0.2 mL of acetic acid (3%) solution was injected intraperitoneally. The number of writhing movements characterized by contraction of abdominal muscles followed by opening back feet or body stretching in the animals were observed and counted immediately and recorded at intervals of 5 minutes for 30 minutes according to method described by [33]. The degree of antinociception (analgesia) was expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with extracts.

2.4.2 Percentage inhibition of acetic acid-induced abdominal writhing

The percentage inhibition of the writhing response was calculated from the equation described by [34] and presented in Figure 1.

$$\text{Percentage (\%)} \text{ writhing inhibition} = \frac{W_c - W}{W_c} \times 100$$

where W_c is the mean number of writhes in the control and W is the mean number of writhes in the extract group.

2.4.3 Formalin-induced paw licking in mice

Formalin test was carried out according to methods by [35;36;37]. Twenty-five adult Swiss albino mice (15-25g) of both sexes were randomized into 5 groups of 5 mice per group. They were fasted for 24 hours but still had water *ad*

libitum. Group I received 10 mL/kg distilled water orally (negative control), group II received 100 mg/kg acetylsalicylic acid (ASA) while groups III, IV and V were pretreated with 474.34mg/kg, 948.68mg/kg and 1423.03mg/kg doses of ethanol leaf extract of *Sphenocentrum jollyanum*. All administrations were done intraperitoneally.

Thirty minutes after treatment of the extract and drug, 0.02 mL of 2.5% formalin solution was injected subcutaneously under the surface of the right hind paw of each mouse. The time durations of licking/ biting of the formalin - injected paw by the mice were observed and recorded for 30 minutes. The time interval between the paw licks was observed and recorded as the index of pain.

2.4.4 Percentage inhibition of formalin-induced paw licking in mice

The percentage inhibition of the paw licking for the acute phase (i.e. 0-5 mins) and chronic (5-30 mins) of the analgesic activity of formalin injection, representing the neurogenic and inflammatory pain response were calculated using the formula:

$$\% \text{ inhibition} = \frac{(Do - Dt)}{Do} \times 100$$

Where Do was the average number of paw licks of the control group, while Dt was the number of paw licks of the treated group. The results are presented in Figure 2

2.4.5 Thermally-induced pain in mice

The methods described by [38,39] was used to determine the effect of thermally-induced pain in this study. Twenty-five (25) mature albino mice (15-20g) of both sexes were randomly divided into 5 groups of 5 mice per group were used for the experiment. The mice were fasted for 24 hours but still received water *ad libitum* before treatment. Group I received 10 mL/kg of distilled water and served as the negative control. Groups II received 4mg/kg body weight dexamethasone (standard drug) while groups III, IV and V were pretreated with 474.34mg/kg, 948.68mg/kg and 1423.03mg/kg of ethanol leaf extract of *Sphenocentrum jollyanum*. All administration was done intraperitoneally.

In this experiment, the hot plate was maintained at $45 \pm 1^\circ\text{C}$ and a glass beaker of 50 cm diameter was placed on the heated surface. The mice were dropped into the beaker and were immediately removed as soon as responses like kicking its hind foot and jumping about, licking and shaking of foot, raising one or the other of the hind foot and holding the foot tightly to its body were noticed as described by [40]. The time it took for the animal to elicit the reflexive pain behaviour was taken as the pain reaction time (PRT) and recorded for each of the animals after the administration of the treatments. A maximum reaction time of 30 seconds was observed to prevent any injury to the tissues of the paws of the mice as described by [41]. Prolongation of the reaction time in the drug treated animals compared to the control indicates analgesic effect.

2.4.6 Percentage inhibition of thermally-induced pain in mice

The percentage increase or decrease in reaction time of thermally-induced pain in mice (as index of analgesia) at each time was calculated from the equation described by [42] and presented in Figure 3.

$$\% \text{ inhibition} = \frac{(Rt)}{Rc} - 1 \times 100$$

where R_t = reaction time in treated group; R_c = reaction time in control group.

2.5 Statistical Analysis

All results are expressed as mean $\pm$ standard error of the mean (SEM) and were analyzed using one-way analysis of variance (ANOVA). Post hoc analysis (comparison across groups) was carried out using the least significant difference (LSD) incorporated into the IBM SPSS software version 20. The values of $p < 0.05$ were considered to be statistically significant

3.0 Results and Discussion

3.1 Acetic acid-induced abdominal writhing

Table 1. Effect of ethanol leaf extract of *Sphenocentrum Jollyanum* on acetic acid-induced writhing in mice.

Group	Treatment	Dosage	5 mins	10 mins	Writhing Time Intervals		25 mins	30 mins	105 mins (Total time)
					15 mins	20 mins			
I.	Distilled Water (mL/kg)	10	22.00 ± 0.95	16.20 ± 0.66	12.60 ± 0.51	7.00 ± 0.32	5.80 ± 0.66	4.00 ± 0.32	65.40 ± 2.1
II	ASA (mg/kg bw)	50	14.00 ± 0.95 ^a	11.00 ± 0.63 ^a	5.40 ± 0.51 ^a	3.00 ± 0.32 ^a	1.80 ± 0.49 ^a	0.40 ± 0.24 ^a	35.60 ± 2.23 ^a
III	<i>S. Jollyanum</i> (mg/kg bw)	474.34	17.40 ± 0.51 ^{a, b}	15.20 ± 0.49 ^b	11.20 ± 0.73 ^b	5.40 ± 0.24 ^{a, b}	3.00 ± 0.32 ^a	2.80 ± 0.20 ^{a, b}	55.00 ± 1.41 ^{a, b}
IV	<i>S. Jollyanum</i> (mg/kg bw)	948.68	16.00 ± 0.55 ^a	12.40 ± 0.51 ^{a, c}	7.00 ± 0.32 ^{a, b, c}	4.00 ± 0.32 ^{a, b, c}	2.80 ± 0.66 ^a	1.20 ± 0.20 ^{a, b, c}	43.40 ± 1.5 ^{a, b, c}
V	<i>S. Jollyanum</i> (mg/kg bw)	1423.03	15.00 ± 0.32 ^{a, c}	12.20 ± 0.37 ^{a, c}	3.20 ± 0.49 ^{a, b, c, d}	3.20 ± 0.20 ^{a, c}	3.00 ± 0.32 ^a	0.80 ± 0.20 ^{a, c}	37.40 ± 0.68 ^{a, c}

Results presented as mean ± S.E.M; n = 5; p < 0.05 is considered significant. a = significant when all test groups is compared with control group 1, b = significant when group 2 is compared with control. c = significant when groups 4&5 are compared with 3. d = significant when groups 4vs5 are compared. ASA: acetylsalicylic acid. bw= body weight.

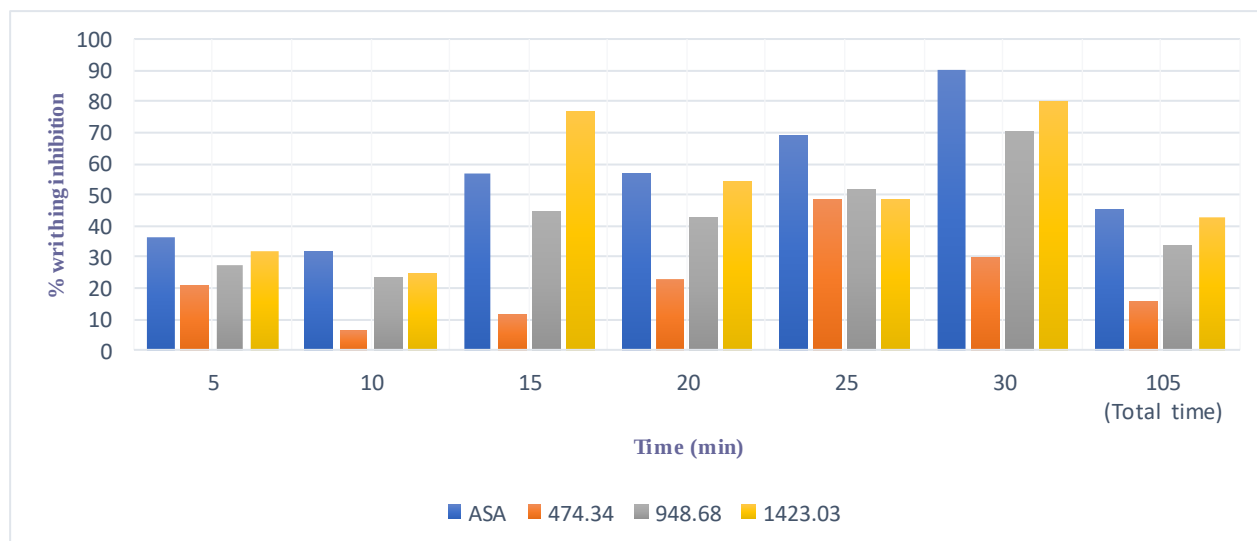


Figure 1: Percentage inhibition of ethanol extract of *Sphenocentrum Jollyanum* on acetic acid-induced writhing in mice.

The *in-vivo* analgesic activity of ethanol extract of *Sphenocentrum jollyanum* leaves using feasible analgesic models such as acetic acid-induced abdominal writhing, formalin-induced paw licking and thermally-induced pain in albino Wistar mice was investigated in this study.

Acetic acid-induced writhing reflex is a visceral pain model nociception which is highly useful for screening analgesic drugs and several chemicals such as phenyl paraquinine, bradykinin and acetic acid that could induce a pain reaction characterized as writhing response reflex in laboratory animals [43]. Various pain mediators such as kinins, acetylcholine, substance P, calcitonin-gene-related peptide and prostaglandins (PG) are also known to initiate writhing reflexes in animals through direct activation of chemo sensitive nociceptors that detect noxious stimuli [44]. Acetic acid-induced writhing model produces pain sensation in animals by triggering an inflammatory response and such pain stimulus leads to the release of arachidonic acid from tissue [45]. Writhing of the abdomen, turning of the trunk (twist) and extension of the hind limbs is considered as some writhing reaction to chemically induced pain in animals [46]. The level of analgesia in acetic acid-induced models is indicated by the percentage reduction in the number of abdominal constrictions [47]. In this study, administration of different doses of ethanol extract of *Sphenocentrum Jollyanum* leaves (474.34, 948.68 and 1423.03 mg/kg) resulted in a dose-dependent significant reduction in the abdominal writhing responses in mice after acetic acid injection compared to the control (Table 1). When all the test groups were compared with control group I, there was significant ($p < 0.05$) decreased abdominal constrictions initiated by the intraperitoneal administration of acetic acid-induced writhing after 105 mins that was highest in acetylsalicylic acid standard drug group II. The highest percentage inhibition (42.8%) was observed at the highest dose and was comparable with acetylsalicylic acid standard drug (45.6%) (Figure 1). This effect may be mediated through peripheral pain mechanism or through suppression of prostaglandin pathway. It has been documented that agents that decreases the number of writhing demonstrate their analgesic effect by the inhibition of prostaglandin synthesis or via peripheral mechanism of pain inhibition [48]. Analgesia activity through peripheral analgesic that inhibits prostaglandin biosynthesis by acting on visceral receptors sensitive to acetic acid has also been reported [49].

3.2 Formalin-induced paw licking

3.2 Formalin-induced paw licking

Table 2 Effect of ethanol leaf extract of *Sphenocentrum Jollyanum* on formalin induced paw licking in mice.

Group	Treatment	Dosage	Paw Licking Time Intervals						
			5 mins	10 mins	15 mins	20 mins	25 mins	30 mins	105 mins (Total time)
I.	Distilled Water (mL/kg)	10	20.20 ± 1.11	15.20 ± 0.49	6.20 ± 0.49	2.80 ± 0.200	9.00 ± 4.58	2.20 ± 0.49	45.00 ± 0.95
II	ASA (mg/kg bw)	100	12.80 ± 0.80 ^a	4.20 ± 1.02 ^a	2.80 ± 0.66 ^a	1.80 ± 0.37	5.00 ± 1.15	0.60 ± 0.24 ^a	24.00 ± 2.97 ^a
III	<i>S. Jollyanum</i> (mg/kg bw)	474.34	14.80 ± 0.66 ^a	13.00 ± 0.32 ^{a,b}	6.00 ± 0.32 ^b	2.40 ± 0.40	2.20 ± 0.20	2.00 ± 0.32 ^b	40.80 ± 0.49 ^b
IV	<i>S. Jollyanum</i> (mg/kg bw)	948.68	14.00 ± 0.63 ^a	8.80 ± 0.66 ^{a, b, c}	5.80 ± 0.20 ^b	2.40 ± 0.80	1.80 ± 0.37	1.80 ± 0.20 ^b	35.00 ± 0.84 ^{a,b,c}
V	<i>S. Jollyanum</i> (mg/kg bw)	1423.03	13.00 ± 0.32 ^a	6.00 ± 0.84 ^{a, c, d}	5.20 ± 0.20 ^b	2.20 ± 0.20	1.20 ± 0.20 ^a	1.00 ± 0.32 ^{a, c}	28.60 ± 0.60 ^{a,b,c}

Results presented as mean ± S.E.M; n=5; p< 0.05 is considered significant. a = significant when all test groups is compared with control group 1, b = significant when group 2 is compared with control. c = significant when groups 4&5 are compared with 3. d = significant when groups 4vs5 are compared. ASA: acetylsalicylic acid. bw = body weight.

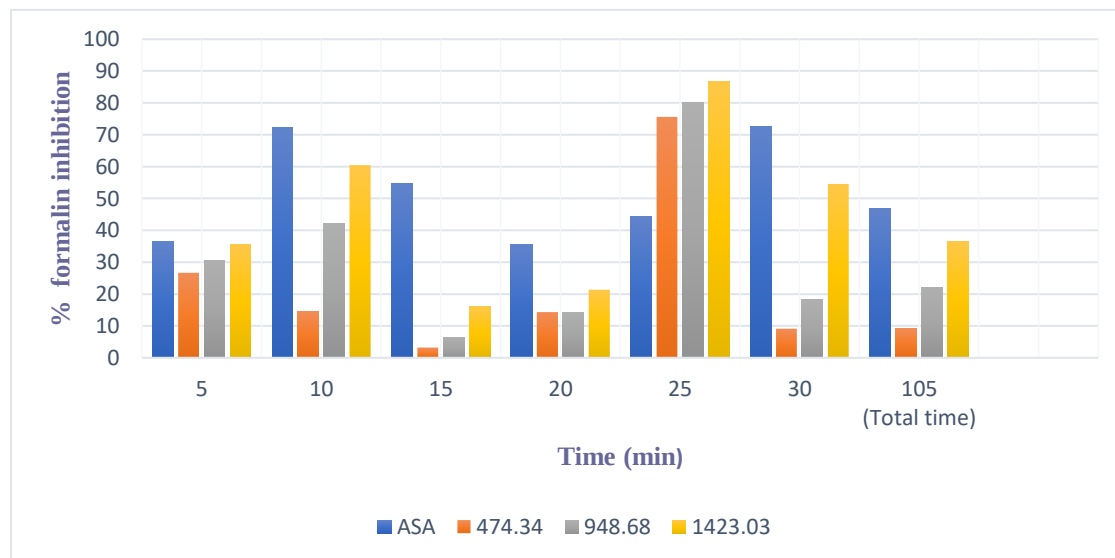


Figure 2: Percentage inhibition of ethanol extract of *Sphenocentrum Jollyanum* on formalin-induced paw licking in mice.

The formalin-induced paw licking model is another method devised to evaluate the analgesic effect of new drugs [50]. Formalin induced possesses two distinct phases reflecting the different types of pain. During the first phase (0–5 min), there is a direct chemical stimulation of the nociceptors (neuropathic or non-inflammatory pain) from chemical stimulation that provoked the release of bradykinin and substance P, whereas the second phase (15 – 30 min) shows the involvement of inflammatory mediators such as histamine, 5- hydroxytryptamine (5-HT), prostaglandins and bradykinin [51]. The injection of formalin in mice has been reported to cause behavioural response such as paw licking, which is a biphasic response [52;53]. The first phase of formalin-induced hind paw licking is centrally mediated [54], while the late phase of formalin-induced hind paw licking is peripherally mediated. The extractability of any agent to inhibit both phases of formalin-induced paw licking has been suggested to be as a result of the inhibition of pain mediators such as bradykinins, substance P, histamine and prostaglandins. This is consistent with the action of centrally acting analgesics while peripherally acting analgesics act only during the second phase of the model [55]. Administration of different doses of the *Sphenocentrum Jollyanum* extract also resulted in a dose-dependent significant ($p < 0.05$) reduction in the formalin-induced paw licking in mice compared with the control group I after 105 mins, that was highest in acetylsalicylic acid standard drug group II (Table 2). Equally, *Sphenocentrum Jollyanum* leaf extract caused a dose-dependent increase in percentage inhibition of paw licking, increasing from 9.3 to 36.4% at total timing compared with standard drug (46.7%) (Figure II). The reduction of the time of paw licking in both phases of measurement indicates that the ethanol extract of *Sphenocentrum Jollyanum* leaf may have the potential to exert anti-nociceptive effects on both inflammatory and non-inflammatory pain. This significant effect of *Sphenocentrum Jollyanum* leaf extract may be probably

mediated via both neurogenic and inflammatory mechanisms. The analgesic effect may be enhanced by its rich presence of phytochemicals such as flavonoids, alkaloids, tannins, and saponins [17]. These phytochemicals may act individually or synergistically. Phytochemicals constituents have been documented to possess significant analgesic and antipyretic activities [56].

3.3 Thermally-induced pain

Table 3 Effect of ethanol leaf extract of *Sphenocentrum Jollyanum* on thermally-induced pain in mice

Group	Treatment	Dosage	Reaction Time (seconds)
I.	Distilled Water	10	16.00 $\pm$ 1.41
II	Dexamethasone (mg/kg bw)	4	48.40 $\pm$ 3.98 ^a
III	S. Jollyanum (mg/kg bw)	474.34	37.60 $\pm$ 4.66 ^{a, b}
IV	S. Jollyanum (mg/kg bw)	948.68	41.40 $\pm$ 3.14 ^a
V	S. Jollyanum (mg/kg bw)	1423.03	46.40 $\pm$ 2.48 ^a

Results presented as mean $\pm$ S.E.M; n =5; p < 0.05 is considered significant. a = significant when all test groups is compared with control group 1, b = significant when group 2 is compared with control. c = significant when groups 4&5 are compared with 3. d = significant when groups 4vs5. ASA: acetylsalicylic acid. bw =body weight.

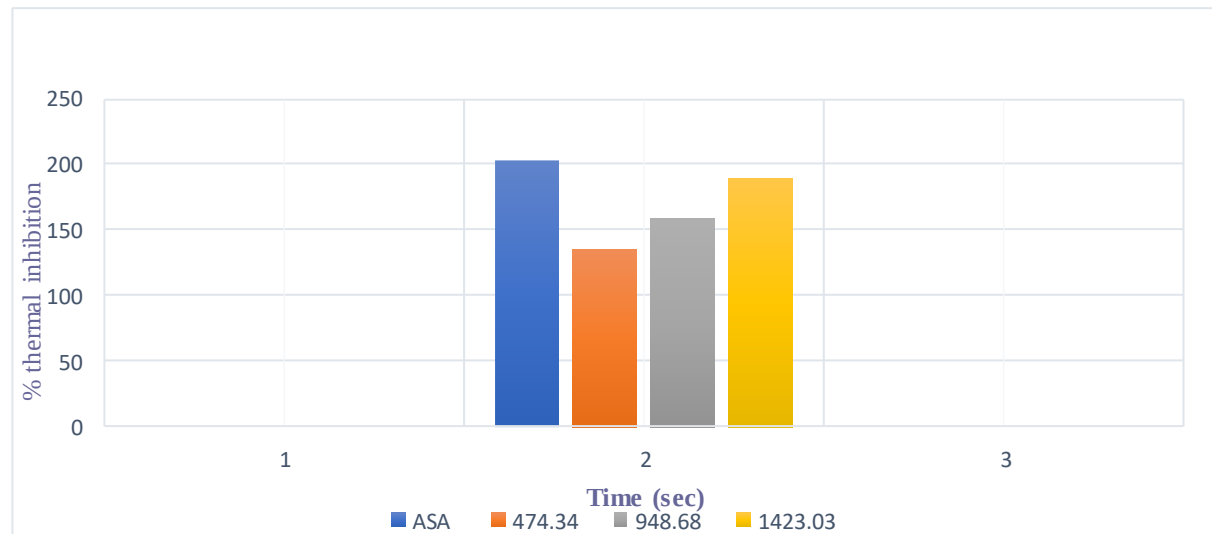


Figure 3: Percentage inhibition of ethanol extract of *Sphenocentrum Jollyanum* on thermally-induced pain in mice.

Sphenocentrum Jollyanum leaf extract showed a significant ($p < 0.05$) increase in the prolongation of pain reaction time (PRT) or latency to thermally induced pain in a dose-dependent manner when compared with control and the highest percentage inhibition was observed with highest dose when compared to other doses (Table 3). This suggest that the leaves of *Sphenocentrum Jollyanum* have prolonged latency period of analgesic activity. Centrally-acting antinociceptive drugs has been reported to increase the pain threshold of animals towards heat and pressure [57]. This centrally acting analgesics exert their analgesia by inhibiting cyclooxygenase pathway of arachidonate metabolism, which produces prostaglandins. These prostaglandins have a variety of effects on blood vessels, nerve endings and cells involved in inflammation [58]. The leaf extract of *Sphenocentrum Jollyanum* may exert this effect by inhibiting this pathway. This maybe further made possible because of its rich phytochemical constituents. A number of alkaloids, saponins, tannins, phlobatannins, flavonoids and cardiac glycosides among others isolated from medicinal plants have been reported to possess significant analgesic activity [59]

4. Conclusion

The result obtained from this study suggest that the leaf extract of *Sphenocentrum jollyanum* may possess significant analgesic activity. The analgesic potential of the extract may be due to the rich phytochemical constituent of the leaf extract such as alkaloids, saponins, tannins, phlobatannins, flavonoids and cardiac glycosides. However, further investigations are required to isolate and characterize the bioactive compounds of extract of *Sphenocentrum jollyanum* leaf and its mechanism of action ascertained.

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