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Evaluation of aqueous-methanol stem bark extract of *Stereospermum kunthianum* Cham. (Family: Bignoniaceae) for Anti-inflammatory and antinociceptive effects

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Abstract

Stereospermum kunthianum Cham (Family; Bignoniaceae) is a plant that has its different parts used in traditional medicine for the treatment of different ailments. Its stem bark extract is used for treatment of wounds, ulcers, gastritis, bronchitis and other pain and inflammatory related health conditions. This study was prompted by the need to authenticate some of these ethnomedicinal claims. The stem bark of *S. kunthianum* was successively macerated in 80 % v/v methanol and the extract was used to evaluate its usefulness in pain and inflammatory conditions. The aqueous-methanol extract of *S. kunthianum* was analysed for phytochemical constituents. Its acute toxicity profile was determined in rats and mice. Anti-inflammatory activity of the extract was evaluated using fresh egg albumin-induced paw oedema model and formalin-induced paw oedema model in rats. The extract was also tested for anti-nociceptive effect using acetic acid-induced writhing test in mice and formalin-induced pain test in rats. The results revealed that saponins, terpenes, tannins and steroids were present in the extract. The estimated oral and intraperitoneal median lethal dose (LD_{50}) of the extract in rats was $\geq 5,000$ mg/kg. The intraperitoneal LD_{50} of the extract in mice was also $\geq 5,000$ mg/kg. The extract (100, 200 and 400 mg/kg i.p) reduced egg albumin-induced paw oedema in rat over a period of 120 min (2 h). The reduction was significant ($p < 0.05$) up to 60 min. and the percent inflammatory inhibition for the extract was comparable to that of acetyl salicylic acid (ASA, 100 mg/kg i.p). Formalin-induced oedema test also revealed that *S. kunthianum* reduced paw oedema in rats up to Day 6 and the reduction effect was higher than that of ASA (100 mg/kg p.o). Acetic acid-induced writhing test for anti-nociception showed that *S. kunthianum* (100 – 400 mg/kg i.p) significantly ($p < 0.05$) reduced the number of acetic acid-induced writhes in mice over 120 min and the reduction was dose-dependent. The extract doses of 200 and 400 mg/kg had higher percent inhibition of nociception than the tested dose of acetyl salicylic acid (100 mg/kg p.o). Anti-nociceptive study also revealed that *S. Kunthianum* (100, 200 and 400 mg/kg i.p) caused a significant ($p < 0.05$) reduction of formalin-induced pain at the late phase (15 – 60 min). The reduction was not dose-dependent and was comparable to that of ASA (100 mg/kg i.p). The extract (100, 200 and 400 mg/kg i.p) produced non-significant pain inhibition in the early phase (0 – 10 min). In conclusion, the study showed the justification for the ethno-medicinal use of *S. kunthianum* stem bark extract for the treatment of inflammatory and pain-related health conditions. The findings are also suggestive of peripheral mechanism of action. *S. kunthianum* stem bark extract therefore has the potential to be developed as anti-inflammatory and analgesic agent.

Introduction

Inflammatory response involves a spectrum of cellular and systemic events that occur in which the host attempts to restore and maintain homeostasis following any one of a variety of tissue injuries; either by mechanical or chemical agents or by self-destruction/auto-immune processes [1, 2]. This therefore shows that, although there is a tendency in clinical medicine to consider the inflammatory response as harmful reaction to the body, it is essentially a 'protective' and 'restorative' response in which the body attempts either to return to the pre-injury condition or to repair itself after inflicted injury [3].

However, if the inflammatory response is 'aberrant', a serious consequence may occur. For instance, an outpouring of too much fluid from the vasculature into an area such as the brain may lead to a serious rise in intracranial pressure.

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The accumulation of fluid due to inflammation in the pleural and pericardial cavities may seriously compromise organ function [4, 5]. Also, the arrival of excessive numbers of neutrophils and the subsequent discharge of their enzymatic contents may result in serious structural damage [6].

It has been shown that many diseases confronting the clinician are due to an uncontrolled inflammatory response. The joint damage in rheumatoid arthritis, the functional and structural damage in glomerulonephritis, and the demyelinating diseases of the central nervous system are examples of excessive or uncontrolled inflammatory response. The treatment usually involves anti-inflammatory therapy since information about the causative agents of these entities is not well known [7, 8].

Pain is on the other hand defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [9] and it is categorized into: 'nociceptive pain' and 'neuropathic pain'. Nociceptive pain is the normal physiological response to a painful stimulus and serves as a biologic function to warn of injury. In the nociceptive pain pathway, there are nociceptors which are free primary nerve endings found in cutaneous muscle and visceral tissues [10]. The nociceptors are normally silent when not stimulated but can be stimulated in two ways: actual injury to the tissue or changes in the tissues surrounding the area of injury. Tissue damage caused by noxious (harmful, injurious) stimulation precipitates cellular changes. The pH changes, enzymes and mediators are activated and released and there may be ionic changes influencing membrane permeability. The inflammatory cascade is stimulated; histamine and serotonin are released increasing vasodilation and inflammation. Some or all of these stimulate the free nociceptor nerve endings [10]. After the stimulation of the nociceptors, the cellular changes in the nerve endings are converted to an electrical impulse in the primary afferent nerve. This impulse continues travelling and ascending to the dorsal or ventral roots of the spinal cord [11].

Neuropathic pain: is on the other hand caused by dysfunction or damage in the nervous system [12] and is an inappropriate response wherein damaged nerves cause signals to travel in abnormal pathways [13, 14].

The type of pain experienced by a patient is directly related to the type of wound although a patient's perception of pain is physiologically decreased by a process of modulation (i.e. The body's method of decreasing pain intensity) by inhibiting the ascending transmission of the pain impulse from the primary afferent neuron to the second order neuron in the spinal cord [10, 15]. It is, however, undeniable that unresolved pain negatively impacts both wound healing and patient's quality of life [12].

Pain is frequently experienced and is multi-dimensional; involving both physiological and psychological components [16]. The physical components include the underlying cause of the pain and pain from clinical interventions. Pain, undoubtedly, increases the amount of stress and anxiety that is perceived by the patient and unaddressed pain can sensitize all parts of the nervous system [17].

Analgesics are agents used to produce diminished sensation to pain without loss of consciousness [18]. Many analgesics already in use have some limitations. Hence, the need for continuous search for new agents that will be efficacious, safe, cheap and readily available.

Ethomedicinal report on *Stereospermum kunthianum* suggested a possible usefulness of its stem bark in the development of anti-inflammatory and/or analgesic agent(s). It is a deciduous shrub or small tree widely spread across Africa with some species distribution in Asia. *S. kunthianum* is found in wooded savanna, bush, rocky outcrops and margins of evergreen forests. The species is well spread all over the Sahel region and is often found near streams [19]. It grows 3-15 m high, with a stem diameter of 25 cm. It has thin, grey-black bark, smooth or flaking in patches resembling the European plane tree; the trunk is rarely straight, mostly forked, with twisted branches. The leaves are impair-pinnately compound, 25 cm long, alternate with 2-6 leaflets. The flowers are precocious (early development), fragrant with mauve to off-white, more usually pinkish with red streaks. The fruits are slender with flat capsules or paired pods [20].

S. kunthianum is commonly called 'Pink Jacaranda' in English. In Nigeria, it is known as dan Sarkin-itatuwa, sansami (Hausa), weknavunihi (Gwari), buldumhi golombi (Fula-fulfude), golombi (Kanuri) umana tumba (TIV), ajade, ayada, afe (Yoruba).

Materials and Methods
Plant Collection and Identification
Fresh plant materials of *Stereospermum kunthianum* stem bark were collected in the month of December from Suleja, Niger State, which is situated at 10°00'N 6°00'E, Nigeria. The plant was identified by a Plant Taxonomist with the Herbarium Unit, Department of Medicinal Plants Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. The specimen was deposited in the NIPRD Herbarium with voucher specimen number: NIPRD. H. 7072.

Preparation of the Plant Extract
The stem bark of *S. Kunthianum* was air-dried and pulverized in a mortar and 1.6 kg (1600 g) of the pulverized sample was macerated successively in 5 litres of 80 % v/v methanol under a temperature of $40^{\circ}C$ on a shaker (GFL D 3006 mgH, Germany) for agitation to ensure maximum extraction. Double maceration was done over a period of 24 hours each. The extract was then filtered using Whatman No 1 filter paper and the filtrate was concentrated using rotary evaporator (KNF RC 900 Neuberger, USA). The concentrate was then placed over a water bath to ensure proper dryness of the extract. The percentage yield of the extract was calculated as follows:

% yield = (W1 X 100)/W2

Where: W1 = Weight of dry extract; W2 = Weight of dry plant

The extract was then stored in the refrigerator (4 °C) for the studies.

Phytochemical Analyses
The extract was screened for the presence or absence of various phytochemical constituents using the standard method [21].

Chemical and Drugs
Analytical grade chemicals and a standard drug were used for the studies and these included Methanol (Fluka Chemie, Switzerland), Glacial acetic acid (Searle, Essex, England), Formaldehyde 40 % w/v (M & B, England), Acetyl salicylic acid (Aspar Pharmaceuticals).

Animals
Wistar rats (125.0 – 256.0 g) of both sexes and Swiss albino mice (15.0 – 30.0 g) of both sexes were used for the studies. They were obtained from the Animal Facility Centre, Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The experimental animals were separated for at least two weeks in the experimental room for acclimatization. The animals were maintained under normal environmental temperature, approximately normal 12 h day and night illumination cycle. They were allowed free access to standard feed and water except when starvation was required in the study.

Acute Toxicity Studies
The modified method of Lorke [22] was adopted for the estimation of the dose of the extract that will cause lethality of 50 % of the animal population (LD_{50}) to which it would be administered to. The study was carried out using oral and intraperitoneal routes in Wistar rats and intraperitoneal route in Swiss albino mice.

The method involves administration of the extract in a biphasic manner. In the first phase, widely differing doses of the extract (10, 100 and 1000 mg/kg) were administered intraperitoneally in the rats and mice to determine the range within which toxicity would occur. The same doses of the extract (10, 100 and 1000 mg/kg) were also administered to rats orally in the first phase. The second phase was dependent on the observations made in the first phase and involved administration of higher doses of the extract (2000, 3000 and 5000 mg/kg) intraperitoneally to new set of experimental rats and mice. The same doses of the extract (2000, 3000 and 5000 mg/kg) were also administered orally to new set of rats in the second phase.

The treated animals were observed for 72 h for behavioural and\or toxic effects such as nervousness, ataxia, excitement, alertness, dullness and death.

Anti-Inflammatory Studies
Stereospermum kunthianum stem bark extract was evaluated for anti-inflammatory activity using acute and chronic anti-inflammatory models as follows:

Egg albumin - induced paw oedema in rats
The study was done according to the modified method of Akah and Nwambie [23]. The rats to be used for the investigation were deprived of water during the experiment to ensure uniform hydration and minimize variability in oedematous response. The rats were then grouped into five (n=5). The first group was given distilled water (10 ml/kg i.p.) to serve as negative control. Three doses of the extract (100, 200 and 400 mg/kg i.p.) were administered to the second, third and fourth groups respectively while acetyl salicylic acid (ASA, 100 mg/kg i.p.) was given to the fifth group to serve as a reference standard.

Inflammation was then induced 30 min post treatment by injecting 0.1 ml of fresh egg albumin into the sub-plantar surface of the right hind paw of each of the rats. The paw thickness of each of the rats was measured using a Vernier caliper (Aerospace, China). Zero readings were taken before the injection of egg albumin (0 min) and at 20 min intervals after the injection of egg albumin over a 2 h (120 min) period. The thickness of the paw at every interval was calculated in relation to the mean paw thickness before the injection of the egg albumin. Activity for the treated groups was expressed as percent inhibition of inflammation in relation to the control group. The percentage inhibition of inflammation was calculated by the formula used by Akah and Nwambie [23] as follows:

Percentage inhibition= $[(C-T)/C) \times 100]$

Where: C= Paw thickness of control rats; T= Paw thickness of test rats

Formalin-induced oedema in rat hind paw
The study was carried out according to the modified method adopted by Shivaji *et al.* [24]. Rats were grouped into five (n=5). Each rat in group one was given distilled water (10 ml/kg p.o.) to serve as negative control. Three doses of the extract (100, 200 and 400 mg/kg p.o.) were administered to the second, third and fourth groups respectively. Rats in group five received acetyl salicylic acid (ASA; 100 mg/kg p.o) to serve as the reference standard.

One-hour post treatment, 0.1 ml of 2 % formalin was injected into the sub-plantar area of the right hind paw of each rat. Treatment with all the drugs was continued for seven (7) consecutive days and treatment was once on each of these days. The paw thickness of each of the rats was measured using a Vernier caliper (Aerospace, China). On the first day, zero readings were taken before the injection of 2 % formalin and then, at 30 min, 1 h, 2 h, 3 h and 4 h post treatment. Readings were also taken once from days 2 – 7.

The thickness of the paw at every interval was calculated in relation to the mean paw thickness before the injection of the 2 % formalin. Activity for the treated groups were expressed as percent inhibition of inflammation in relation to the control group. The percentage inhibition of inflammation was calculated using the formula described by Shivaji *et al.* [24] as follows:

Percentage inhibition = $[(C-T)/C) \times 100]$

Where: C = Paw thickness of control rats; T= Paw thickness of test rats

Anti-Nociceptive Studies
These studies involved evaluation of the extract for anti-nociceptive activities and determination of the possible site(s) of anti-nociception if there is anti-nociception.

Acetic Acid-induced Writhing Test in Mice
This involved a test on chemical pain and the method used by Chidume *et al.* [25] was adopted. Swiss albino mice of either sex were used for the investigation. They were grouped into five (n=5). Distilled water (10 ml/kg i.p) was administered to the first group. The second, third and fourth groups received graded doses of the extract (100, 200, 400 mg/kg i.p.) respectively. Acetyl salicylic acid (ASA; 100 mg/kg i.p) was administered to the mice in group five to serve as the reference standard.

At intervals of 30, 60, 90 and 120 min post administration, 0.75 % glacial acetic acid was administered intra-peritoneally to each mouse at the dose of 10 ml/kg. Five minutes after acetic acid injection, the number of writhes made by each mouse within 10 min was counted using a counter.

The percent writhes for the treated group was calculated in relation to the control group. The activity was expressed as percent inhibition of nociception (reduction in episodes of writhing between the negative control and the treated groups).

Formalin-induced pain test in rats

This procedure shows the possible site(s) of anti-nociception and was done according to the method used by Jegede *et al.* [26]. Adult wistar rats of either sex were used. They were grouped into five (of five rats each). Distilled water (10 ml/kg i.p) was given to rats in group one to serve as negative control. The extract (100, 200, 400 mg/kg i.p) was given to rats in groups two, three and four respectively. Acetyl salicylic acid (100 mg/kg i.p) was administered to the rats in group five to serve as reference standard.

Thirty (30) min post treatment, 50 µl (0.05 ml) of 2.5 % formalin was injected under the plantar surface of the left hind paw of each rat. The rats were then placed under observation.

The severity of pain was recorded as scores

(0) = rat walked or stood firmly on the injected paw
(1) = rat partially elevated or favoured the paw
(2) = rat elevated the paw from the floor
(3) = rat licked, bit or shook the paw.

The cut off points for the observations was every 2min for the first 10 min (early phase) and at every 5min for the period between the 10th and 60th min (late phase).

Data Analyses

The results of the studies were expressed as mean ± SEM. The differences among the treatment groups were analyzed using Analysis of Variance (ANOVA) in the SPSS version 16 software. Tukey Post hoc Test was used to determine the differences between treatment groups. P-values < 0.05 were taken to be statistically significant. Results were presented as a table and diverse charts as appropriate.

Results

Plant Extract

The weight of the pulverized *S. kunthianum* stem bark was 1600.0 g (1.6 kg) while the weight of *S. kunthianum* extract was 279.0 g giving a percentage yield of 17.44 % (w/w). The extract was dark brown in appearance but oily and slurry in consistency.

Phytochemical Analyses

The phytochemical analyses carried out on the crude stem bark extract showed the presence of saponins, terpenes, tannins and steroids

Acute Toxicity Studies

No overt toxicity signs or death was observed in rats 72 h post oral treatment with *S. kunthianum* extract (10 – 5,000 mg/kg). The estimated oral median lethal dose (LD_{50}) of the extract in rats was therefore ≥ 5,000 mg/kg.

No death was observed in rats and mice 72 h post intraperitoneal treatment with S. kunthianum extract (10 – 5,000 mg/kg). No overt toxicity sign was observed in rats while mice were calm within the first 20 min. post administration with the extract (3,000 – 5,000 mg/kg i.p). The estimated intraperitoneal median lethal dose (LD50) of the extract in rats and mice was therefore ≥ 5,000 mg/kg.

Anti-inflammatory Studies

Egg albumin-induced paw oedema in rats

The stem bark extract of *S. kunthianum* (100, 200, 400 mg/kg i. p) reduced rat paw thickness over a period of 120 min (2 h). The reduction was significant ($p < 0.05$) up to 60 min. The effect was not dose-dependent and was comparable to ASA (100 mg/kg i.p; Figure 1). The percentage inhibition of inflammation for *S. kunthianum* extract (100, 200, 400 mg/kg i.p) was 26.2 %, 23.8 %, 33.3 % respectively. The percent inhibition for the extract was comparable to that of acetyl salicylic acid (ASA, 100 mg/kg i.p) with percent inhibition of 24.6 %.
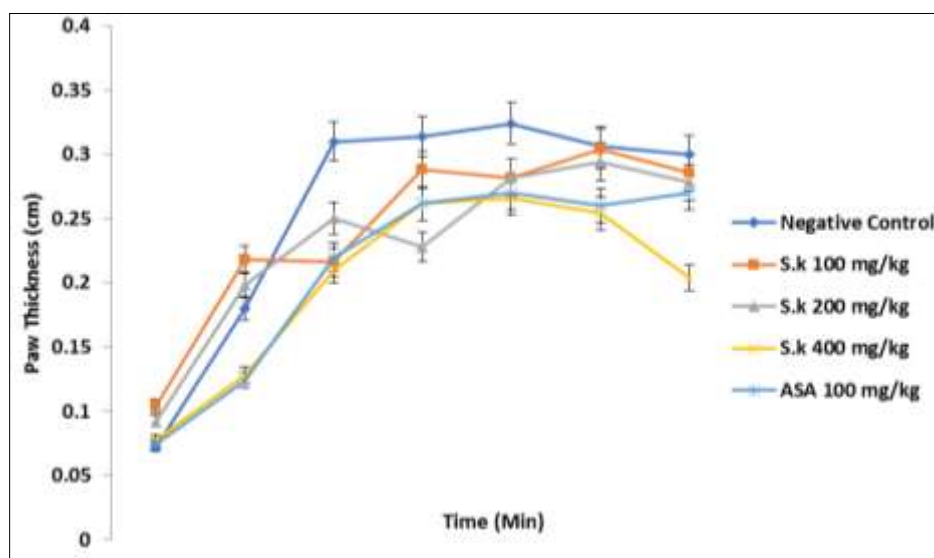

Fig 1: Effect of aqueous-methanol extract of *S. kunthianum* stem bark (S.K) on fresh egg albumin-induced paw oedema in rats

Formalin-induced Oedema in rat hind paw

The extract of *S. kunthianum* (100 mg/kg p.o) reduced rat paw thickness up to Day 6. The reduction was significantly ($p<0.05$) different from the negative control up to Day 3. On the other hand, *S. kunthianum* extract (200 – 400 mg/kg p.o) caused a non-significant reduction of the rat paw thickness up to Day 4.

The paw thickness reduction by the extract (100 – 400 mg/kg) was higher than that of ASA (100 mg/kg p.o) which had a non-significant reduction of the rat paw thickness up to Day 4 (Figure 2). The general percentage inhibition of inflammation for *S. kunthianum* extract (100, 200, 400 mg/kg i.p) was 17.7 %, 19.9 %, 21.8 % respectively.
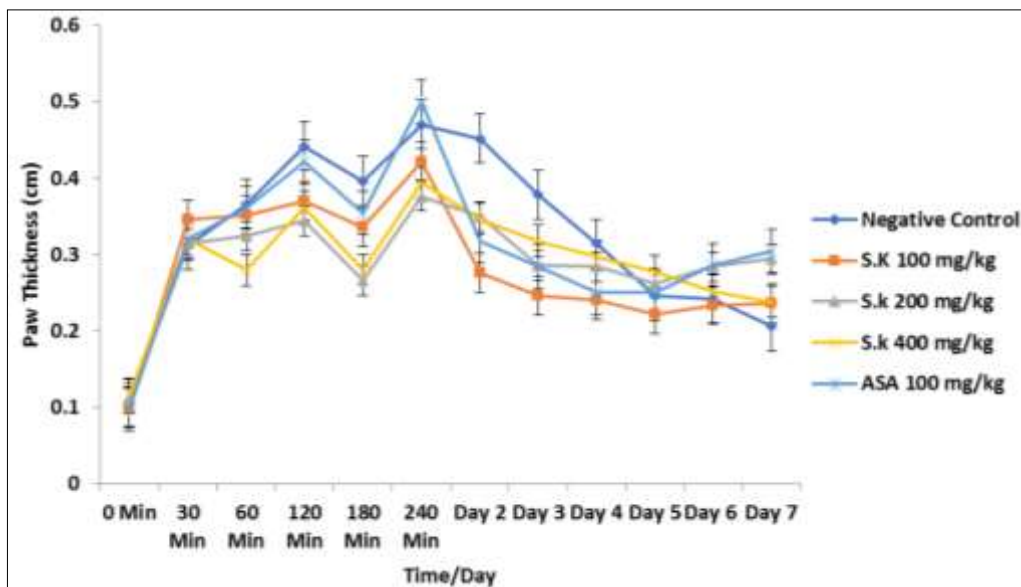
Fig 2: Effect of aqueous-methanol extract of *S. kunthianum* stem bark (S.K) on formalin-induced paw oedema in rats

Anti-Nociceptive Studies
Acetic Acid-induced Writhing Test in Mice
The stem bark extract of *S. kunthianum* (100 – 400 mg/kg i.p) significantly ($p<0.05$) reduced the number of acetic acid-induced writhes in mice over the 120 min test period. The reduction effect was dose-dependent. ASA (100 mg/kg i.p) also caused a significant ($p < 0.05$) reduction of the writhes.

However, *S. kunthianum* (200 – 400 mg/kg i.p) significantly ($p<0.05$) reduced the number of writhes more than ASA (100 mg/kg i.p; Figure 3). The percent inhibition of nociception for *S. kunthianum* (100, 200 and 400 mg/kg i.p) was 58.8 %, 80.7 % and 97.4 % respectively. The extract doses of 200 and 400 mg/kg showed higher percent inhibition of nociception than acetyl salicylic acid with percent inhibition of 67.9 %.
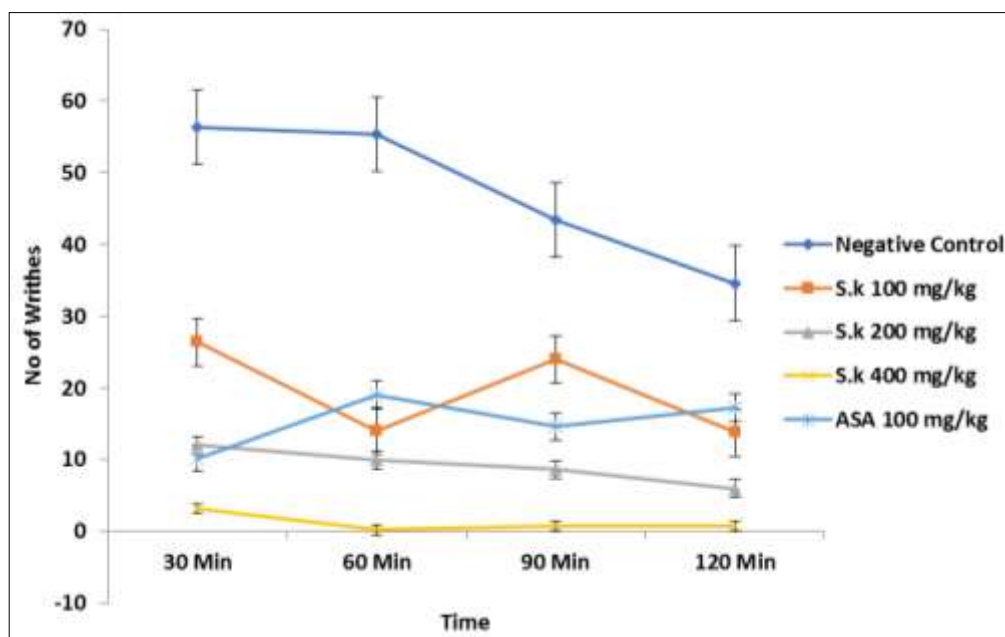

Fig 3: Effect of aqueous-methanol extract of *S. kunthianum* stem bark (S.K) on acetic acid-induced writhes in mice

Formalin-induced Pain Test in Rats
In the early phase (0 – 10 min), the rats in the negative control group showed mean pain score of 11.6 (equivalent to 100.0% pain inhibition) while the aqueous-methanol extract of *S. kunthianum* (100, 200 and 400 mg/kg i.p) had mean pain scores of 11.4, 10.6 and 11.8 which are equivalent to 1.72 %, 8.60 % and -1.72 % percent pain inhibition respectively. The rats treated with ASA (100 mg/kg i.p) showed mean pain score of 12.6 (equivalent to -8.62 % pain inhibition) in the early phase. In the late phase (15 – 60 min), however, the negative control rats had mean pain score of 24.6 (equivalent to 100.0% pain inhibition). The aqueous-methanol stem bark

extract of *S. kunthianum* (100, 200 and 400 mg/kg i.p) caused a significant ($p < 0.05$) but non dose-dependent reduction of formalin-induced pain at the late phase (15 – 60 min) with mean pain scores of 12.8,15.0 and 13.6 giving percent pain inhibition of 47.97 %, 39.02 % and 44.72 % for the respective doses of the extract. The frequency by which the aqueous-methanol *S. kunthianum* (100 – 400 mg/kg i.p)-treated rats licked, bit or shook the paw was markedly reduced between 15 – 60 minutes. This result was comparable to that of ASA (100 mg/kg i.p) with mean pain score of 43.09 which is equivalent to percent pain inhibition of 43.09 % at the late phase (15 – 60 min; Table 1).

Table 1: Effect of aqueous-methanol extract of *S. kunthianum* stem bark (100, 200, 400 mg/kg p.o) on formalin-induced pain in rats

Treatment	Early Phase		Late Phase	
	Score of pain	% Pain Inhibition	Score of pain	% Pain Inhibition
Negative Control	11.6 ± 0.9	0.00	24.6 ± 1.2	0.00
S. kunthianum				
100 mg/kg i.p.	11.4 ± 1.2	1.72	$12.8 \pm 2.2*$	47.97
200 mg/kg i.p	10.6 ± 1.0	8.60	$15.0 \pm 0.9*$	39.02
400 mg/kg i.p	11.8 ± 1.3	-1.72	$13.6 \pm 2.6*$	44.72
ASA				
100 mg/kg i.p	12.6 ± 1.0	-8.62	$14.0 \pm 1.0*$	43.09

Values are expressed as mean $\pm$ SEM (n = 5); $*P < 0.05$, significantly different from the control; One-way ANOVA; Tukey post hoc.

Discussion

Acute toxicity study was used to establish the median lethal dose (LD_{50}) of stem bark extract of *S. kunthianum* in mice and rats treated orally and intraperitoneally. The evaluation was useful in the determination of the working doses for the study and for the classification of the plant extract in terms of safety. According to Lorke [22], different substances have different toxicity levels, hence, the classification of substances into very toxic, toxic, less toxic or only slightly toxic. This indicates that evaluation of safety profile of a drug is paramount in the development of drugs and in their subsequent clinical uses.

The present study showed that the stem bark extract of *S. kunthianum* has estimated oral and intraperitoneal $LD_{50} \geq$ 5,000 mg/kg in rats and intraperitoneal LD_{50} in mice. The $LD_{50} \geq 5,000$ mg/kg estimated for rats and mice in this study indicates relative safety since Lorke [22] considered LD_{50} values greater than 1g (1000 mg/kg) for a test substance or chemical as only slightly toxic (relatively safe).

The oral acute toxicity value $\geq$ of 5,000 mg/kg obtained in this study for stem bark extract of *S. kunthianum* corroborated the oral LD_{50} value of $\geq 8,000$ mg/kg obtained by Ching *et al.* [27]. These results suggest that the stem bark extract of *S. kunthianum* is relatively safe.

It should, however, be noted that, although acute toxicity study (LD_{50}) is useful, such acute toxicity data are of limited clinical application since cumulative toxic effects do occur even at very low doses. Hence, sub-acute and chronic toxicity studies are almost always invaluable in evaluating the safety profile of phytomedicines [28]. This is probably the basis for the suggestion that sub-chronic toxicity data be used to predict the hazard of long term, low-dose exposure to a particular compound [29].

In the present study, egg albumin-induced oedema study revealed that the percent inhibition was not dose-dependent and was comparable to that of acetyl salicylic acid (ASA, 100 mg/kg i.p) with percent inhibition of 24.6 %. This could mean that the precursors or mediators of the inflammatory process were only minimally attenuated at these tested doses. However, the highest percentage inhibition effect (33.3 %) recorded at the highest dose (400 mg/kg) is indicative that the anti-inflammatory activity of the *S. kunthianum* stem bark extract may increase as its doses increase. Although the mechanism of action for inflammatory inhibition by *S. kunthianum* stem bark extract was not elucidated in the present study, the presence of saponins may have contributed to the anti-inflammatory effect. Navarro *et al.* [30] reported that saponins have anti-inflammatory activity which can reduce oedema and skin inflammation.

The minimal percent inhibition of inflammation (26.2 %, 23.8 % and 33.3%) exhibited by the extract (100, 200, 400 mg/kg respectively) might just have been adequate enough to prevent aberrant inflammatory response. The stem bark extract exhibited inhibitory effect at all the tested doses with the

highest effect at the highest dose. The dose to be used clinically would therefore be dependent on the degree of inflammation. It implies that doses can be increased as the degree of inflammation increases.

The results for the formalin-induced oedema in rat hind paw corroborated those of egg albumin-induced oedema. It showed percentage inhibition of inflammation by *S. kunthianum* extract (100, 200, 400 mg/kg p.o) to be 17.7 %, 19.9 % and 21.8 %. This also suggests that increasing doses of the *S. kunthianum* extract might also increase the inhibitory effect on inflammation. The study also revealed that *S. kunthianum* (100 mg/kg p.o) reduced oedema up to Day 6. This reduction was significant ($p < 0.05$) up to Day 3. Furthermore, *S. kunthianum* (200 and 400 mg/kg p.o) caused a non-significant reduction of rat paw oedema up to Day 4. This suggests that *S. kunthianum* stem bark extract has the potential to be developed as an anti-inflammatory agent for both acute and sub-acute inflammation. Therapeutic advantage can be taken of this property in the management of chronic wounds. This is in consideration that chronic wounds are characterized by a chronic inflammatory response which impedes healing [31]. This anti-inflammatory effect may be attributable to the presence of saponins in the stem bark extract [30].

The study also revealed that *S. kunthianum* stem bark extract (100 – 400 mg/kg i.p) significantly ($P<0.05$) and dose-dependently reduced the number of acetic acid-induced abdominal constrictions (writhes) in mice. This probably suggests an anti-nociceptive property. This effect progressed over the 120 min. (2 h) observation period suggesting a possible prolongation of anti-nociception effect. The *S. kunthianum* stem bark extract (200 – 400 mg/kg i.p) significantly ($P <0.05$) reduced the number of writhes than acetyl salicylic acid (ASA; 100 mg/kg i.p). The percent inhibition of nociception for *S. kunthianum* stem bark extract (100, 200, and 400 mg/kg i.p) was 58.8 %, 80.7 % and 97.4 % respectively. The extract dose rates of 200 and 400 mg/kg showed higher percent inhibition of nociception than acetyl salicylic acid (ASA; 100 mg/kg i.p) which had percent inhibition of 67.9 %. However, the stem bark extract at the dose of 100 mg/kg i.p showed lower percent inhibition of nociception than acetyl salicylic acid. This is an indication that *S. kunthianum* stem bark extract has the potential of being developed into analgesic with comparable effects as those of acetyl salicylic acid.

The use of abdominal constriction (writhing) model for detection of anti-nociceptive activity has been reported to be more sensitive, when compared with other models such as tail flick model [32]. The writhing response is thought to partly involve local peritoneal receptors [33, 34, 35].

In the present investigation, formalin test was adopted to elucidate the possible site(s) (central, peripheral, or both) of anti-nociceptive activity observed in the extract. The results revealed that *S. kunthianum* extract (100, 200 and 400 mg/kg

i.p) significantly ($p < 0.05$) reduced formalin-induced pain in the late phase (15 – 60 min) of the experiment. The frequency by which *S. kunthianum* extract (100, 200 and 400 mg/kg i.p) – treated rats licked, bit or shook the paw was markedly reduced between 15 – 60 minutes and their scores for pain were also reduced at the late phase. However, the reduction was not dose-dependent with percent pain inhibition of 47.97 %, 39.02 % and 44.72 % for the respective doses of the extract. The result was comparable to that of ASA (100 mg/kg i.p) with percent inhibition of 43.09 % at the late phase (15 – 60 min). On the other hand, *S. kunthianum* extract (100, 200 and 400 mg/kg i.p) produced percent pain inhibition of 1.72 %, 8.60 % and – 1.72 % respectively in the early phase (0 – 10). These effects were not different from that of the negative control group with percent inhibition of 0.00 %.

Dubuisson and Dennis [36] and Tjolsen *et al.* [37] reported that in formalin test, nociception occurs in two phases. The first phase starts immediately after formalin injection and continues for 5 min, after which nociception appears to diminish. The second phase is marked by a return to high levels of nociception beginning 15 – 20 min. after formalin injection and continuing for 60 min. The first phase is probably a direct result of stimulation of nociceptors in the paw, while the second phase may reflect the inflammation process, and at least to some degree, the sensitization of central nociceptive neurons [38, 39]. This method is very useful for elucidating the mechanism of pain and analgesia [37].

Drugs such as narcotics which act mainly centrally, inhibit both phases of formalin-induced pain while drugs, such as aspirin, hydrocortisone and dexamethasone which are primarily peripherally acting only inhibit the late phase [40, 41, 42]. The lower percent pain inhibition of 17.2 %, 8.60 % and - 1.72 % recorded at the early phase (0 -10 min) and the higher percent pain inhibition of 47.97 %, 39.02 % and 44.72 % recorded at the late phase (15 - 60 min) show action of the extract preferentially on the late phase and suggests that the peripheral mechanism may be involved.

The higher pain inhibition percentage in the second phase of formalin test indicates peripheral anti-inflammatory process and suggests peripheral mechanism of pain relief. The earlier report on the anti-inflammatory effect of the extract on fresh egg albumin-induced oedema and formalin-induced oedema corroborate this. It can therefore be deducted that the peripheral mechanism may be the major mechanism involved in the anti-nociceptive effect of *S. kunthianum* stem bark extract. This could therefore mean that the extract may be of the antipyretic analgesic type rather than the opioid analgesic. Subsequent study will involve the interaction of the anti-nociceptive action of the extract with opioid antagonists such as naloxone, to see if the effect could be reversed as is typical of opioid analgesics.

It is important to note that some drugs such as phenacetin, acetaminophen (paracetamol) are known to be clinically effective analgesics, even antipyretics but lack significant anti-inflammatory properties while other drugs such as phenylbutazone are potent anti-inflammatory agents but lack or have only weak analgesic properties. Others have both analgesic and anti-inflammatory properties e.g. acetyl salicylic acid (aspirin). The present investigation has shown that *S. kunthianum*stem bark extract inhibited egg albumin-induced oedema (inflammation), formalin-induced oedema (inflammation), acetic acid-induced writhes (chemical pain test) and formalin-induced pain showing. Although the antipyretic activity of the extract is yet to be evaluated, it is possible that the extract belongs to the same group as acetyl salicylic acid (aspirin) which has analgesic and anti-inflammatory properties.

In conclusion, the results corroborate the ethnomedicinal use of *S. kunthianum* stem bark extract for the treatment of inflammatory and pain-related health conditions. *S. kunthianum* stem bark extract therefore has the potential to be developed as anti-inflammatory and analgesic agent.

Authors' Contributions
Prof. Paul Abdu and Dr Shehu NaAllah Alhaji Saidu played supervisory and quality assurance roles in the study. Prof. Florence Chimezie Nwinyi was the Principal Investigator and she worked in collaboration with Dr Joseph Omamegbe. Mr Adamu Mohammed provided the technical assistance.

Availability of Data and Materials
All the relevant data and materials have been presented in the manuscript

Ethical Approval
Approval of research protocol was obtained from University of Abuja Ethics Committee on Animal Use (UAECAU) with reference number: UAECAU/2018/006. The research was conducted according to the internationally accepted principles for laboratory animal use and care of the NIH publication no. 85-23.

Consent for Publication
Not applicable

Conflict of Interests
No conflict of interest was declared by the authors.

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