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Research Article

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Evaluation of Aqueous Methanol Stem Bark Extract of *Stereospermum kunthianum* (Pink Jacaranda) for Analgesic and Anti-Inflammatory Effects in Alloxan-Induced Diabetic Rats

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Abstract

The stem bark extract of Stereospermum kunthianum (Family: Bignoniaceae) also known as pink jacaranda is used in traditional medicine to treat rheumatic arthritis, other inflammatory and pain-related health conditions in humans. The aim of this study was to evaluate the analgesic and anti-inflammatory properties of the stem bark extract of S. kunthianum (Pink Jacaranda) in alloxan-induced diabetic rats for the purpose of drug research and development. The study is also aimed at finding an alternative treatment using medicinal plants to arrest pain and inflammation which are associated with delayed wound healing common in diabetic patients. The stem bark was successively macerated in 80% v/v methanol to obtain the extract. The aqueous-methanol extract of S. kunthianum was analysed for various phytochemical constituents. The aqueous-methanol stem bark extract of the plant was evaluated for 72 h for its acute toxicity in rats. Diabetes was induced in the healthy adult Wistar albino rats by injecting alloxan at a dose of 115 mg/kg body weight. The aqueous-methanolic extracts of Stereospermum kunthianum was administered at doses of 100, 200, 400 mg/kg body weight intraperitoneally. The aqueous-methanol extract of the stem bark was also investigated for anti-inflammatory effect in the diabetic rats using fresh egg albumin-induced paw oedema. The analgesic effect of the extract at the doses of 100, 200 and 400 mg/kg was tested against acetic acid-induced nociception and formalin-induced pain in diabetic rats. The results revealed that saponins, terpenes, tannins and steroids were present in the extract. Its acute toxicity profile was determined in the diabetic rats. In the acute toxicity studies, there was no observed toxicity sign up to the dose up to 5000 mg/kg p.o. The estimated oral median lethal dose (LD50) of the extract was ≥ 5,000 mg/kg. The stem bark extract of Stereospermum kunthianum (100, 200 or 400 mg/kg, i.p.) caused a reduction of paw oedema in the diabetic-induced rats over a period of 120 min (2 h). However, the extract at all the test doses (100, 200, 400 mg/kg i.p.) caused a significant ($p<0.05$) reduction of inflammation only at 60 min observation period in comparison with the control group (distilled water: 10 ml/kg, i.p.) The effect of the extract was most pronounced at the dose of 100 mg/kg, i.p. and was higher at 80 min and 120 min than that of acetyl salicylic acid (50 mg/kg, i.p.). The percent inflammatory inhibition for the extract was not dose-dependent and was comparable to that of acetyl salicylic acid (ASA, 50 mg/kg i.p.). Acetic acid-induced writhing test for anti-nociception showed that S. kunthianum stem bark extract (100, 200, 400 mg/kg, i.p.) significantly ($p <0.05$) reduced the number of acetic acid-induced writhes in diabetic rats for 180 min in a time-dependent manner and the reduction was not dose-dependent. The extract at a dose of 200 mg/kg had higher percent inhibition of nociception than that produced by doses of 100 and 400 mg/kg of the stem bark extract. Acetylsalicylic acid (ASA, 50 mg/kg, i.p.) maintained a steady analgesic effect > 80% from the onset (30 min) to termination of the study (180 min) post pain induction in the diabetic rats. At 90, 120 and 180 min, 200 mg/kg, i.p. stem bark extract of S. kunthianum displayed an almost equipotential analgesic effect comparable to ASA (50 mg/kg, i.p.) in the diabetic rats. In the formalin-induced pain test in the diabetic rats, the S. kunthianum extract (100, 200 mg/kg, i.p.) had minimal analgesic potency at both doses in the early phase compared to acetylsalicylic acid (ASA, 50 mg/kg, i.p.) with a high analgesic potency in early phase. The highest dose of stem bark extract (400 mg/kg, i.p.) produced non-significant pain inhibition in the early phase (0 –15 min). 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 (30-45 min) with ASA having a minimal analgesic effect compared to the test doses. The doses of the stem bark extract of S. kunthianum (100, 200, 400 mg/kg, i.p.) had weak analgesic effects in both phases of formalin-induced pain model. This suggests that the analgesic effect of the stem bark extract of S. kunthianum involves both neurogenic (centrally) and inflammatory (peripheral) mechanism. The findings of the study indicate that the aqueous extract of Stereospermum kunthianum stem bark possesses anti-inflammatory activity which is probably related to the inhibition of prostaglandin synthesis. The paw edema, acetic-acid writhing and formalin findings showed that the stem bark extract of Stereospermum kunthianum possess significant anti-nociceptive and anti-inflammatory effects. These findings justify the folkloric use of the stem bark extract of S. kunthianum in ethnomedicine for the management of various painful and inflammatory conditions.

Keywords: Stereospermum Kunthianum Stem Bark Extract, Phytoconstituents, Acute Toxicity, Diabetic Rats, Anti-Inflammatory Activity, Paw Odema, Acetic-Acid Writhing Test, Formalin Test

1. Introduction

The main problem faced by individuals with diabetes is that wounds take longer time to heal and are often accompanied by excruciating pain and inflammation. The inability of the body to combat infection, heightened inflammation of cells, and discomfort are all linked to diabetes. When a wound occurs, the first reaction of the skin is inflammation in response to the injury [1]. Inflammation is a defensive reaction in the body triggered by various factors such as irritation, infection, or tissue damage. It involves the release of inflammatory mediators to eliminate the irritating substance or microorganism and facilitate the healing process of the affected tissue [2-4]. The process of inflammation is a constantly changing process, in which proinflammatory cytokines like tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and vascular endothelial growth factor (VEGF) have important functions [5]. The inflammatory response can be stimulated by physical trauma, toxic chemicals, microbial invasion, and hypersensitive reactions. Currently, there is a significant global health concern due to the rise in inflammatory diseases such as arthritis, cardiovascular disease, and diabetes mellitus. These conditions have become a leading cause of increased deaths annually [6]. Painful diabetic neuropathy (PDN) is a severe outcome of diabetes that can affect up to 20% of individuals with diabetes. Moreover, roughly 16 to 34% of individuals diagnosed with diabetes experience painful symptoms related to nerve damage, with a higher occurrence in type II diabetes [7,8]. Diabetic neuropathy is a common condition that impacts around 50% of individuals with both type 1 and type 2 diabetes. It is linked to health complications, such as foot ulcers and pain. Inflammation is commonly linked to various diseases and numerous traditional medicine practitioners in Nigeria receive significant support and achieve great success in addressing this issue. Although many different types of plants are used for this specific purpose, there is limited scientific and pharmacological information available about them. However, individuals who have diabetes often experience ongoing pain and inflammation. These issues can become the main concern in their care, diverting attention away from managing diabetes effectively [9-12]. Stereospermum kunthianum holds great significance to local communities due to its numerous uses. The ethnomedicinal study on Stereospermum kunthianum from the Bignoniaceae family reveals that rural residents in Nigeria use this plant to alleviate febrile convulsions, rheumatic arthritis, and various other inflammatory and painful ailments [13]. This implies that the stem bark may have potential of reducing inflammation and pain. The plant *Stereospermum kunthianum* is commonly referred to as pink jacaranda, a deciduous shrub or small tree native to Africa, specifically found in the Democratic Republic of Congo, Nigeria, and various other regions of the continent. *Sterospermum kunthianum* can reach a diameter of 25 cm when it grows. Its bark is thin, and it has a gray-black color that appears smooth or peels off in patches, similar to the bark of a London plane tree. The trunk of this tree is usually not straight and has twisted branches. Typically reaching a height of 5 meters, although sometimes as tall as 15 meters, the tree is known for its impressive display of numerous, fragrant, early blooming pink or purplish flowers, creating a stunning visual of the plant [14].

2. Objectives

The objectives of the study are to: identify the phytochemical constituents present in the stem bark extract of *S. kunthianum* and to determine the median lethal dose (LD_{50}). To determine the acute toxicity of the extract and establish a safe dosage for the research, the median lethal dose of the extract was tested. The goal was to find a working dose that would not result in any deaths among the experimental rats. In order to assess the pain-relieving effects of the *S. kunthianum stem bark extract in diabetic rats,* the acetic acid -induced writhing method and formalin test was performed. Furthermore, the anti-inflammatory properties of the aqueous-methanol stem bark extract of *S. kunthianum* using egg albumin-induced paw oedema test was evaluated in the diabetic rats. Therefore this investigation will provide scientific justification for the use of *S. kunthianum stem bark* in traditional medicine to treat and manage rheumatic arthritis, as well as other inflammatory and pain-related health conditions.

3. Materials and Methods

3.1 Plant Collection and Identification

The fresh stem bark of *Stereospermum kunthianum* was collected by the author from Apia village in Suleja district of Niger State, situated at $10^{0}00'$N $6^{0}00'$E, Nigeria. This was done during the rainy season specifically between May and June and in accordance with the Good Field collection practices by NMPB. The research was conducted at the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Abuja, Nigeria. The plant sample was identified by a plant Taxonomist, Mr. Muazzu, employed at the Herbarium Unit, Department of Medicinal Plants Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. The plant's authenticity was confirmed by comparing it to relevant voucher specimens. Afterwards, the samples were then deposited in the Pharmacology laboratory at the University of Abuja, located in Nigeria.

3.2 Preparation of Plant Extract

After being collected, the stem bark of Stereospermum kunthianum was rinsed with water and subsequently fragmented using a knife into smaller pieces. Next, the cut pieces of the stem bark of S. kunthianum was left to dry in the air for a period of 12 days and subsequently crushed into powder using a mortar and pestle. The pulverized sample weighing 1.60 kilograms was macerated in 5 liters of 80% v/v methanol solution at a temperature of 40 degrees Celsius. The maceration process involved using a shaker from Germany (GFLD 3006 mgH) to agitate the solution and ensure optimal extraction. Two rounds of maceration were carried out, each lasting for 24 hours. The resulting extract was then passed through a filter paper of Whatman size 1. The liquid that passed through the filter was made more concentrated by using a rotary evaporator from KNF Neuberger, a company based in the USA. Next, the solution was placed on top of a container filled with hot water to make sure that the extract was adequately dried. The stem barks extract's

percentage yield was determined by using the following formula:

$$\% \text{ Yield} = \frac{W1}{W2} \times 100$$

Where W1 = weight of dry extract after extraction; W2 = weight of the stem bark before extraction. The extract was stored in a closed container, kept in a refrigerator at 4°C for subsequent studies.

3.3 Phytochemical Analyses

The phytochemical screening of crude extract for the presence of various secondary metabolites was done using the standard method of Trease and Evans [15]. The extract was screened qualitatively for the presence or absence of various chemical constituents like saponins using the froth test, Libermann-Buchard test for terpenes, ferric chloride test for tannins, Salkowski test for steroids, Aluminum chloride test for flavonoids, Borntrager's test for anthraquinones Molisch test for carbohydrates and Dragendorff's test for alkaloids.

3.4 Phytochemical Screening for Saponins Using the Froth Test

A 2 ml of filtered sample was added to 4 ml of distilled water in a test tube. It was mixed well and shaken vigorously. Foam was produced after ten minutes which persisted on constant shaking; this confirms the presence of saponins.

3.5 Phytochemical Screening for Terpenes Using the Libermann-Buchard Test

A 1ml of the extract was added to chloroform, acetic anhydride and few drops of H2SO4 in a test tube and vigorously shaken. The formation of red-violet color indicates the presence of terpenoids.

3.6 Phytochemical Screening for Tannins Using the Ferric Chloride Test

A 1 ml of the extract was diluted in distilled water and added 2 drops of ferric chloride in a test tube. A greenish-black precipitate indicates the presence of tannins.

3.7 Phytochemical Screening for Steroids Using the Salkowski Test

This was performed by adding 2 ml of chloroform and 1 ml of concentrated sulfuric acid in a test tube. Then, 10 drops of the extract was dissolved in isopropyl alcohol, which was slowly added into the test tube until double phase formation and it was shaken. A reddish-brown color in the middle layer of the test tube is indicative of the presence of steroids.

3.8 Phytochemical Screening for Flavonoids Using the Aluminum Chloride Test

A 4 ml of the extract was added to 1 ml of 1% aluminum chloride solution in a test tube; it was shaken and observed for light yellow coloration. A yellow precipitate indicates the presence of flavonoids.

3.9 Phytochemical Screening for Anthraquinones Using the Borntrager's Test

A 4 ml of the extract was added to 4 drops of diluted sulphuric acid, and then it was boiled for 5 min and filtered and cooled. Then 4 drops of benzene was added to the cooled filterate and was shaken, and then aqueous ammonia was added and shaken very well. The formation of pink-red precipitate indicates the presence of anthraquinones.

3.10 Phytochemical Screening for Carbohydrates Using the Molisch Test

A 2 ml of the extract was added to 1 ml of concentrated sulphuric acid through the sides of test tube to form a layer without shaking. A purplish-reddish colour at the interface of the two liquids revealed the presence of carbohydrates.

3.11 Phytochemical Screening for Alkaloids Using the Dragendorff's Test

This screening was performed by adding 1 ml of Dragendorff's reagent to 2 ml of the extract; an orange red precipitate was formed, indicating the presence of alkaloids.

4. Chemicals and Drugs

Methanol (Fluka Chemie, Switzerland), Alloxan Monohydrate (Sigma Aldrich, USA), Accu-Chek active test strips (mg/dl: Indiana USA), Acetylsalicylic acid (Aspirin: Bayer Bitterfeld GmbH, Philippines), Xylazine (20 mg/ml: Bioverta Czech Republic), Ketamine Hydrochloride injection (Jawa Ketamine, India), Vitamin C (Ascorbic acid; Emzor Pharmaceutical Industries Ltd., Lagos Nigeria), Acetic acid (Sigma Aldrich, USA), Formalin (2.5%: Asta Chemicals, Malaysia) were used for the studies. All chemicals and reagents used were of analytical grade.

5. Experimental Animals

In this experimental study, fifty healthy adult Wistar rats of both sexes weighing (150-200 g) of approximately the same age (4-6 weeks of age) were used for the studies. The rats were procured from the Animal Facility Centre, Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRID), Abuja. The adult Wistar rats were grouped into 5 groups of 5 rats each (n=5). The experimental rats were housed in stainless steel cages for at least two weeks in the experimental room of the Laboratory Animal Unit of the Faculty of Veterinary Medicine, University of Abuja, Nigeria; at room temperature of (26˚C - 27˚C) and under a normal 12 h light/dark illumination cycle (lights on 07:00 am-19:00 pm) daily. The rats were housed for a period of 2 weeks prior to the commencement of the experiments for acclimatization. They were allowed access to standard feed and water *ad libitum* throughout the experimental period except when starvation was needed in the course of the experiment. To prevent the influence of circadian rhythm, all tests were conducted between 8:00 am and 11:00 am. The reason rats were chosen is because they share genetic, biological, and behavioral traits with humans, making them a suitable model for studying human conditions as many symptoms can be reproduced in rats.

6. Ethical Approval

The experimental protocol using the rats was reviewed and approved by the Ethical Committee on Animal Use (UAECAU) of the Faculty of Veterinary Medicine, University of Abuja Nigeria with reference number: UAECAU/2020/0002. The

animal handling and care was in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council [16].

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

7. Acute Toxicity Study

The research used Lorke's method with some modifications [17]. The approach was utilized to determine the quantity of the *S. kunthianum* extract required to cause the death of half of the rats (50%) that were treated (known as the median lethal dose or LD_{50}) after 72 hours. The research was performed on healthy adult Wistar rats, and it involved administering the stem bark extract of S. kunthianum orally. The research was divided into two distinct phases (first and second phase). Nine rats were divided into three groups (groups A, B, and C: n=3) with each group containing three rats. The initial weights of the rats were also recorded. The remedy made from the stock sample was carefully divided and mixed evenly for each group. The rats received the correct dosage based on their weight and were then monitored for 72 hours to count the number of deaths. In the first phase of the research, groups A, B, and C were given the oral stem bark extract of *S. kunthianum* at different doses of (10, 100, and 1000 mg/kg) and their reactions were monitored. No deaths were reported after 72 hours. During the second phase of the research, a new group of nine adult Wistar rats were divided into three groups, with each group consisting of one rat (a total of three rats). Groups A, B, and C were administered the extract at doses of 2000, 3000, and 5000 mg/kg, respectively, using the oral route following the same procedure outlined for the first phase. After the rats received treatment, the rats were monitored for a period of 72 hours to check for any possible changes in their behavior or signs of toxicity. These changes included signs of nervousness, lack of coordination, increased activity, heightened awareness, reduced alertness, and mortality. The lethal dose 50% of the extract was determined by using the formula:

Where D0 = Highest dose that gave no mortality, and D100 = Lowest dose that produced mortality. All the rats that survived were further monitored for two weeks for toxic effects [18].

8. Induction of Diabetes Mellitus Using Alloxan Monohydrate and Blood Glucose Monitoring

Alloxan monohydrate from (Sigma Aldrich Inc. USA) was used to induce diabetes mellitus in the rats. The drug was prepared for injection by dissolving it in distilled water at a concentration of (20 mg/ml) Fifty (50) adult Wistar rats weighing (150-200 g) were fasted for 24 h (depriving them access to feed and water), after which each rat was injected with alloxan monohydrate. The fasting blood sugar of the rats was measured before the alloxan injection. Alloxan monohydrate at (115 mg/ kg) was administered using the intraperitoneal route to each rat. The rats resumed normal feeding after the induction. The blood glucose level in mmol/L of each rat was measured after 48 h of induction using a glucometer by using the tail tipping blood sample technique [19]. Blood was drawn from the tail of each rat, spotted on glucose test strips and the baseline blood glucose levels of the rats were obtained using Accu-chek glucometer and glucose test strips. Rats with blood glucose of ≥ 11 mmol/L were considered diabetic. Diabetes in rats is frequently defined as fasting blood glucose above 7 mmol/L (126 mg/dL; 20,21). The rats with diabetes were later utilized for additional investigations. The model was employed to provoke and sustain the development of type II diabetes. Before the study began, a pilot study for the suitable dose range of alloxan was experimented. When rats were given a high dose of 130 mg/kg of alloxan, a toxic glucose substitute, it resulted in the death of many of the rats. However, a lower dose of 110 mg/kg did not lead to the development of diabetes in the rats. During the experiment, rats were given alloxan monohydrate at a dosage of 115 mg/ kg. This dose caused the rats to develop diabetes, but did not result in any deaths. Based on these findings, it can be concluded that the 115 mg/kg dosage was considered the safest and most effective for this particular experiment. Alloxan monohydrate can be administered through the intraperitoneal, intravenous, and subcutaneous methods. The intraperitoneal method leads to a slight increase in blood sugar levels and no deaths were observed. This is in contrast to the intravenous route, as it leads to a sudden increase in blood glucose levels in the rats, resulting in a high number of recorded deaths. In the case of subcutaneous administration of alloxan monohydrate, the majority of the rats did not develop diabetes.

9. Anti-Inflammatory Studies in Diabetic Rats

The egg albumen-induced paw oedema model was used to evaluate the anti-inflammatory properties of the stem bark extract of *Stereospermum kunthanium* [20].

10. Egg Albumin-induced Paw Oedema in Diabetic Rats

The egg albumen-induced paw oedema model was used to evaluate the anti-inflammatory properties of the stem bark extract of *S. kunthanium.* The anti-inflammatory activity of the stem bark extract was determined according to modified method of Akah and Nwambie [21]. Twenty-five healthy adult diabetic rats (150 -200 g) of both sexes were used for the study. The rats were randomly allocated to five groups (A-E), of five rats each. Distilled water (10 ml/kg i.p.) was administered to the first group (group A) and serve as negative control. Three doses (100, 200, 400 mg/kg, i.p.) of the stem bark extract of *S. kunthianum* were administered orally to the second, third and fourth groups of rats, respectively. Acetylsalicylic acid (ASA; 50 mg/kg, i.p.) was given to the fifth group and serve as the positive control group. Linear paw circumferences of the rats were determined using vernier caliper, before injection of the egg albumin (zero readings). This zero readings were baseline values at the 0 min. Thirty minutes post treatment; oedema was induced by injection of 0.1 ml dose of freshly prepared egg albumin into the sub-plantar region of the left hind paw of each rat. The paw circumference of each rat was measured with the aid of vernier calipers. The readings were taken at 20, 40, 60, 80, 100 and 120 min after the injection of egg albumin. The percentage inhibitory activity was calculated in accordance with the formula [22].

$$\% \text{ inhibition of oedema} = \frac{(Co) \text{ control} - (Ct) \text{ test}}{(Co) \text{ control}} \times 100$$

Where C_o = mean paw size in control group, C_t = mean paw size in treated group. The percentage oedema of each treatment group was compared with that of the negative control.

11. Anti-Nociceptive Studies in Diabetic Rats

Acetic acid writhing reflex test and the formalin-induced pain test were used to evaluate the analgesic properties of *S. kunthianum* stem bark extract.

12. Acetic Acid-induced Writhing Test in Diabetic Rats

The writhing test was performed using a slightly modified method described by Cidade *et al,* [23]. Twenty-five alloxan-induced diabetic rats were grouped into five groups, of five rats each. Distilled water (10 ml/kg IP) was administered to the first group and serve as negative control. Three doses (100, 200, 400 mg/kg) of the stem bark extract of *S.kunthianum* were administered orally to the second, third and fourth groups of rats, respectively. Acetylsalicylic acid, ASA (50 mg/kg i.p.) was given to the fifth group and serve as the positive control group. Thirty minutes after administration of all treatments, each rat was injected with 0.6% acetic acid (10 ml/kg i.p) intraperitoneally and placed in an observation box. At 30, 60, 90, 120, 180 minutes after acetic acid injection, the number of writhing responses of each rat was counted every at 5 minutes interval up to 5 times and was recorded.

The percentage pain inhibition was calculated as follows

$$\% \text{ Pain inhibition} = \frac{Wc - Wt}{Wc} \times 100$$

Where, W is the number of writhing, c is the negative control, and t is the test.

13. Formalin-Induced Pain Test in Diabetic-Induced Rats

The method of Tjolsen *et al* [24]. was used for this study. Twenty - five adult diabetic rats (150 - 200 g) of both sexes were used for this study. The rats were allotted into five groups (n=5) labelled A-E. Group A which serves as the negative control received distilled water (10 ml/kg i.p.). Three doses (100, 200, 400 mg/kg) of the stem bark extract of *S. kunthianum* were administered intraperitoneally to the groups B, C, D respectively. Group E which serves as positive control received acetylsalicylic acid (50 mg/kg i.p.). Each rat received an intra-plantar injection of 0.05 ml of 2.5% formalin (40% formaldehyde) into the dorsal surface of the right hind paw of each rat and was immediately transferred to transparent glass viewing chamber for observation. The nociceptive response was observed and recorded visually for 0-15 mins (Early phase) every 2 minutes and 30-45 mins (Late phase) every 5 minutes. The severity of the pain was scored for the two distinct phases: 0-15 min (Early phase) and 30-45 min (Late phase) post formalin injection was recorded using the pain score from 0-3, as follows; 0, nomal weight bearing on the injected paw of the rat (foot flat on the floor with all toes displayed), 1; light resting of the paw on the floor with no toes display indicating mild pain; 2; elevation of the injected paw and the heel not in contact with any surface, indicating moderate pain, 3; licking, biting, shaking and grooming of injected paw, indicative of severe pain [25,26].

14. Data Analysis

IBM SPSS Statistics version 23 was used for the statistical analyses. The results of the studies were expressed as mean + SEM. The data generated from the studies were analysed using One-way Analysis of Variance (ANOVA) where appropriate. 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 tables, figures and plates as appropriate.

15. Results

15.1 Plant Extract

The weight of the pulverized *S. kunthianum* stem bark was 1600.0 g, while the weight of *S. kunthianum* extract was 279.0 g. The percentage yield was 17.44%. The extract was dark brown in colour, oily and slurry in consistency. Four known compounds which include saponins, terpenes, tannins and steroids were isolated from the aqueous methanol extract of the stem bark of *Stereospermum kunthianum*.

15.2 Phytochemical Analysis

The phytochemical analyses carried out on the crude stem bark extract showed the presence of saponins, terpenes, tannins and steroids. Other compounds which include anthraquinones, carbohydrates and flavonoids were not present in the extract (Table 4.1).

Chemical Constituents	Inference
Anthraquinones	-
Carbohydrates	-
Flavonoids	-
Saponins	+
Steroids	+
Tannins	+
Terpenes	+

Table 4.1: Phytochemical constituents of aqueous-methanol extract of *S. kunthianum* stem bark extract

Key: + = Present; - = Absent

16. Acute Toxicity Studies

The results of the acute toxicity tests with *S. kunthianum* stem bark extract are shown in Table 4.2. No toxic signs or death was observed in the rats 72 h after oral treatment with *S. kunthianum* stem bark extract at doses between 10 and 5,000 mg/kg. The estimated oral median lethal dose (LD_{50}) of the extract in rats was therefore ≥ 5,000 mg/kg. The experimental rats did not exhibit acute signs of toxicity or death within 72 h even at the maximal test dose of 5000 mg/kg following oral of *S. kunthianum* stem bark extract. The oral median lethal dose (LD_{50}) of the extract in rats was estimated therefore to be equal or greater than 5,000 mg/kg.

Treatment	Number of dead rats	Number of rats alive
***S.kunthianum* (p.o.) Phase I**		
10 mg/kg	0/3	3/3
100 mg/kg	0/3	3/3
1000 mg/kg	0/3	3/3
Phase II		
2000 mg/kg	0/3	3/3
3000 mg/kg	0/3	3/3
5000 mg/kg	0/3	3/3

Table 4.2: Acute toxicity study of *Steroespermum kunthianum* stem bark extract in rats

Key: 0/3 =Number of animals which died/number of animals used; 3/3 = Number of animal alive/number of animal used. $LD_{50} \geq$ 5,000 mg/kg

17. Egg Albumin-Induced Paw Oedema in Diabetic Rats

The stem-bark extract of *S. kunthianum* caused a reduction of the paw oedema of the diabetic-induced rats at doses of 100, 200, and 400 mg/kg, i.p. over a period of 120 min (2 h). However, the extract at all the test doses (100, 200, 400 mg/kg i.p.) caused a significant ($p<0.05$) reduction of inflammation only at the 60th minute in comparison with the negative control group (distilled water: 10 ml/kg, i.p.). The reduction effect was most effective at 100 mg/kg, i.p of the extract. The positive control, ASA (50 mg/kg, i.p.) also caused a reduction of the paw oedema throughout the observation period of 2 h (120 min). There was no significant difference observed in the 100, 200 and 400 mg/kg doses of the extract throughout the test period when compared with the positive control group (Figure 4.1, Table 4.3). Twenty minutes after treatment, the extract doses of *S. kunthianum* at 100, 200 and 400 mg/kg inhibited the rat paw oedema by 28.8%, 28% and 29% respectively, compared to distilled water (25.2%) and ASA (26.6%). After 40 minutes of treatment, the extract at 100, 200 and 400 mg/kg inhibited rat paw oedema by 9.7%, 8.6% and 6.7% respectively, compared to -11.1% and 6.8% produced by distilled water and ASA (50 mg/kg), respectively. After 60 minutes of treatment, the stem bark extract of *S. kunthianum* at 100, 200 and 400 mg/kg of inhibited rat paw oedema by 18.1%, 14.3% and 11% respectively, compared to -16% and 19.5% produced by distilled water and ASA (50 mg/kg) respectively. After 80 minutes of treatment, *S. kunthianum* stem bark extract at 100, 200 and 400 mg/kg inhibited rat paw oedema by 24.3%, 21.4% and 16.6% respectively, compared to distilled water and ASA which produced 4.8% and 23.3% respectively. After 100 minute of treatment, the extract doses of 100, 200 and 400 mg/kg inhibited the rat paw oedema by 31.3%, 23.6% and 20.7% respectively, compared to distilled water and ASA which produced 17% and 21% respectively. At 120 minutes after treatment, the extract doses of *S. kunthianum* at 100, 200 and 400 mg/kg inhibited rat paw oedema by 38.2%, 32.9% and 22.8% respectively, compared to distilled water ASA (50 mg/kg) which produced 19% and 34.6% respectively. In the present study, egg albumin-induced oedema study revealed that the percent inhibition was not dose-dependent for the extract doses of 100, 200, 400 mg/kg, the highest percentage inhibition effect (38.2 %) was recorded at the dose of (100 mg/kg, i.p.) and was comparable to that of acetyl salicylic acid (ASA, 100 mg/kg i.p) with percent inhibition of 34.6 %. (Figure 4.1, Table 4.3).

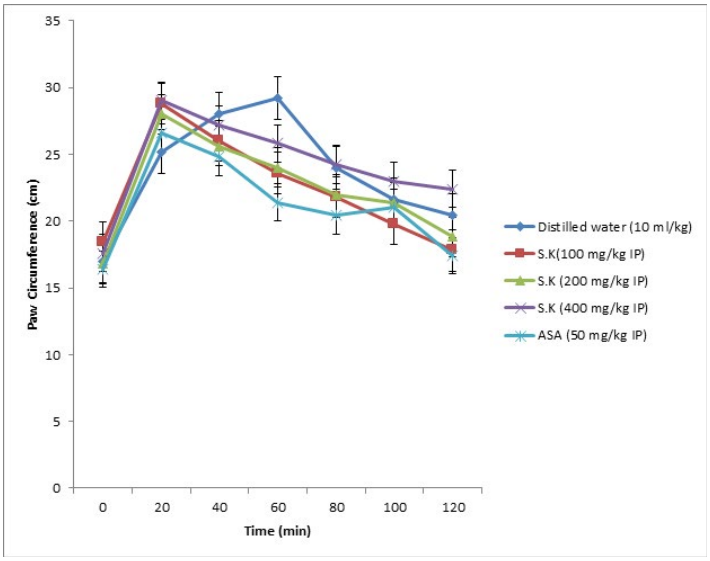

Figure 4.1: Effect of aqueous-methanol extract of Stereospermum kunthianum stem bark on egg albumin-induced paw oedema in diabetic rats.
S.K = Stereospermum kunthianum; ASA= Acetyl salicylic acid; n=5

	Paw thickness Circumference (cm) (%Inhibition of Paw Odema)						
Treatment	**0 min**	**20 min**	**40 min**	**60 min**	**80 min**	**100 min**	**120 min**
Distilled water 10 ml/kg i.p. *S. kunthianum*	17.0 + 1.3	25.2+0.6 (25.2)	28.0+0.4 (-11.1)	29.2+ 0.4 (-16.0)	24.0+ 1.1 (4.8)	21.6+0.9 (17.0)	20.4+ 1.4 (19.0)
100 mg/kg	18.4 + 0.9	28.8+2.1 (28.8)	26.0+ 1.7 (9.7)	23.6+1.3* (18.1)	21.8+1.0 (24.3)	19.8+0.4 (31.3)	17.8+ 0.7 (38.2)
200 mg/kg	16.8 + 0.9	28.0+1.0 (28.0)	25.6+0.8 (8.6)	24.0+0.6* (14.3)	22.0+0.5 (21.4)	21.4+0.7 (23.6)	18.8+ 1.0 (32.9)
400 mg/kg	17.6 + 0.7	29.0+1.0 (29.0)	27.2+1.0 (6.2)	25.8+1.0* (11.0)	24.2+1.5 (16.6)	23.0+ 1.3 (20.7)	22.4+1.0 (22.8)
ASA 50 mg/kg i.p.	16.4 + 0.6	26.6+1.0 (26.6)	24.8+1.1 (6.8)	21.4+ 0.7 (19.5)	20.4+0.7 (23.3)	21.0+0.4 (21.0)	17.4+0.9 (34.6)

Table 4.3: Effect of aqueous methanol Stereospermum kunthianum stem bark extract on egg albumin induced paw oedema in diabetic rats

Values are expressed as mean + SEM (n=5); $p < 0.05$ = significantly different from the control; One-way ANOVA; Turkey post hoc; ASA- Acetylsalicylic acid

18. Acetic Acid-induced Writhing Test in Diabetic Rats

The stem-bark extract of *S. kunthianum* at 100, 200, 400 mg/kg i.p. decreased the acetic acid-induced writhes in a time-dependent manner. All the doses of the extract caused a significant reduction ($P<0.05$) of pain at 30, 90, 120 and 180 min. The effect was highest at 200 mg/kg i.p. Acetylsalicylic acid (ASA; 50 mg/kg i.p.) significantly ($P<0.05$) reduced the number of writhes from 30 min to the 180 min in comparison with the negative control (distilled water: 10 ml/kg; Figure 4.2, Table 4.5). The stem bark extract of *S. kunthianum* (100, 200, 400 mg/kg i.p.) decreased the acetic acid-induced writhes in a time-dependent manner in the diabetic rats. Acetylsalicylic acid (ASA: 50 mg/kg, i.p.) maintained a steady analgesic effect > 80% from the onset (30 min) to termination of the study (180 min) post pain induction in the diabetic rats. At 90, 120 and 180 min, 200 mg/kg, i.p stem bark extract of *S. kunthianum* displayed an almost equipotential analgesic effect comparable to ASA (50 mg/kg, i.p.), the reference drug in the diabetic rats (Figure 4.2). Thirty minutes post treatment, the extract at 100, 200, and 400 mg/kg reduced pain in diabetic rats by 60.4%, 81.3% and 60.4% respectively when compared to 0% produced by distilled water (10 ml/kg). The reduction in pain caused by ASA (50 mg/kg) was 82.6%, thirty minutes after treatment. Similarly, at 60 minutes post treatment, extract at 100, 200 and 400 mg/kg reduced pain in diabetic rats by 35.3%, 49.6% and 55.4% respectively, compared to distilled water and ASA with 0% and 93.5% respectively. Ninety minutes post treatment, the extract at 100, 200 and 400 mg/kg reduced pain in diabetic rats by 67.2%, 82.8% and 71.3% respectively, compared to distilled water and ASA with 0% and 86.9% respectively. One hundred and twenty minutes post-treatment, the extract at 100, 200 and 400 mg/kg reduced pain in diabetic rats by 81.5%, 83.9% and 87% respectively, compared to distilled water and ASA with 0% and 87% respectively. At the end of the study (180 min), extract at 100, 200 and 400 mg/kg reduced pain in diabetic rats by 90.5%, 96.8% and 89.5% respectively, compared to 0% produced by distilled water and 96.8% caused by ASA after 180 minutes (Table 4.4, Table 4.5).

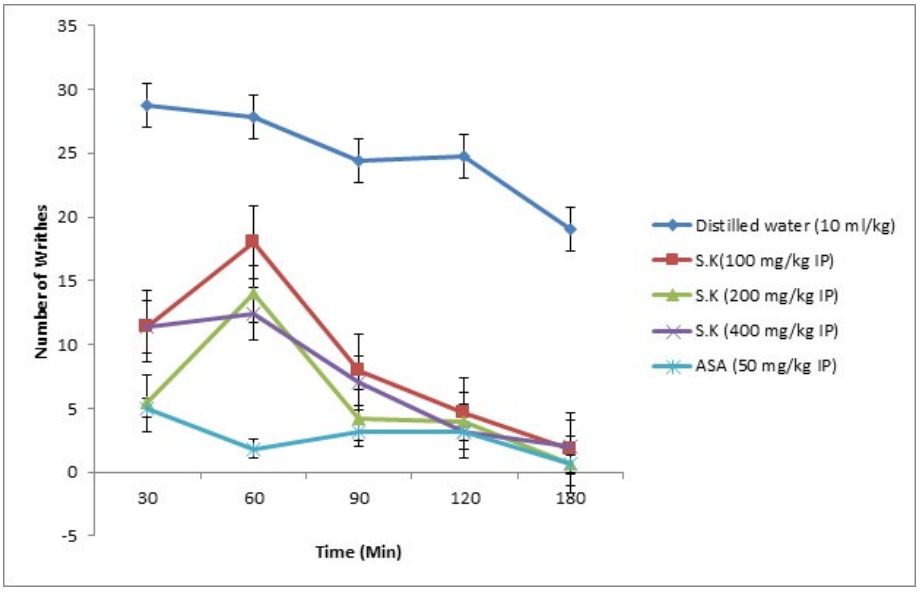

Figure 4.2: Effect of aqueous-methanol extract of *Stereospermum kunthianum* stem-bark on acetic acid-induced writhing in diabetic rats. S.K =
Stereospermum kunthianum; ASA= Acetylsalicylic acid; n=5

	Number of writhes----------				
Treatment	**30 min**	**60 min**	**90 min**	**120 min**	**180 min**
Distilled water 10 ml/kg i.p. *S. kunthianum*	28.8 + 3.7	27.8 + 2.6	24.4 + 3.3	24.8 + 1.8	19.0 + 1.3
100 mg/kg i.p.	11.4 +3.2*	18.0 + 5.2	8.0 + 3.6*	4.6 + 1.0*	1.8 + 1.2*
200 mg/kg i.p.	5.4 + 1.7*	14.0 + 5.3	4.2 + 0.7*	4.0 + 1.4*	0.6 + 0.4*
400 mg/kg i.p.	11.4 + 0.5*	12.4 + 3.8	7.0 + 2.1*	3.2 + 2.2*	2.0 + 0.8*
ASA 50 mg/kg i.p.	5.0 + 2.5*	1.8 + 1.0*	3.2+ 1.2*	3.2 + 1.4*	0.6 + 0.6*

Table 4.4: Effect of aqueous methanol extract of *Stereospermum kunthianum* stem bark on acetic acid-induced writhing test in diabetic rats

Values are expressed as mean + SEM (n=5) ;p<0.05=significantly different from the control; One –way ANOVA. Tukey post hoc; ASA- Acetylsalicylic acid

	Percentage Pain Inhibition (%)				
Treatment	**30 min**	**60 min**	**90 min**	**120 min**	**180 min**
Distilled water 10 ml/kg *Sterospermum kunthianum*	0.0	0.0	0.0	0.0	0.0
100 mg/kg i.p.	60.4*	35.3	67.2*	81.5*	90.5*
200 mg/kg i.p.	81.3*	49.6	82.8*	83.9*	96.8*
400 mg/kg i.p.	60.4*	55.4	71.3*	87.0*	89.5*
ASA 50 mg/kg i.p.	82.6*	93.5*	86.9*	87.0*	96.8*

Table 4.5: Effect of aqueous methanol extract of Stereospermum kunthianum stem bark on percentage pain inhibition of acetic acid-induced writhing test in diabetic rats

* indicates significant differences at p<0.05 compared to the control; ASA- Acetylsalicylic acid

19. Formalin-induced Pain Test in Diabetic Rats

In the early phase of the formalin-induced pain test, 100 and 200 mg/kg of the extract of *S. kunthianum* produced lower pain inhibition of 25.0% and 33.3% respectively compared to 83.3% with ASA (50 mg/kg, i.p.) in the diabetic rats. The *S. kunthianum* extract (100, 200 mg/kg i.p.) had minimal analgesic potency at both doses in the early phase compared to the reference drug (ASA, 50 mg/kg i.p.) with a high analgesic potency in early phase.The *S. kunthianum* stem bark extract (400 mg/kg i.p.) produced non-significant pain inhibition in the early phase (0 –15 min). The result 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 (30-45 min) compared to ASA (50 mg/kg, i.p.) which have a minimal analgesic effect. Pain inhibition with ASA, 100, 200 and 400 mg/kg were 24.1%, 6.9%, 20.7% and 27.6% respectively in the late phase. However, the reduction was in dose-dependent manner for 100, 200, 400 mg/kg of the stem bark extract of *S. kunthianum* at the late phase of the pain test. The stem bark extract of *S. kunthianum* (400 mg/kg i.p.) exhibited a higher analgesic properties in the late phase. The doses of the stem bark extract of *S. kunthianum* (100, 200, 400 mg/kg, i.p.) had weak analgesic effects in both phases of formalin-induced pain model (Table 4.6).

	Early Phase		Late Phase	
Treatment	**Score of pain**	**% Pain Inhibition**	**Score of pain**	**% Pain Inhibition**
Distilled water 10 ml/kg i.p.	2.40+0.6	0.0	2.90+0.1	0.0
S. kunthianum 100 mg/kg, i.p.	1.80+0.5	25.0	2.70+0.1	6.9
200 mg/kg, i.p.	1.60+0.4	33.3	2.30+0.5	20.7
400 mg/kg, i.p.	2.40+0.6	0.0	2.10+0.3	27.6
ASA 50 mg/kg i.p.	0.40+0.2*	83.3	2.20+0.3	24.1

Table 4.6: Formalin-induced Pain Test in Diabetic rats

Values are expressed as mean + SEM (n=5); p<0.05=significantly different from the control; One-way ANOVA; Tukey post hoc; ASA- Acetylsalicylic acid

20. Discussion

The *S. kunthianum* stem bark is used traditionally in managing inflammation and pain-related conditions in humans. The present research was conducted to investigate the potential analgesic and anti-inflammatory effects in a diabetic animal model experiencing pain and inflammation using the stem bark extract of *S. kunthianum*. The stem bark extract of *S. kunthianum* was examined for its phytochemical composition. The phytochemical analyses of the stem bark extract of *S. kunthianum* revealed the presence of saponins, terpenes, tannins, and steroids. The phytochemical compounds of alkaloids, flavonoids, anthraquinones and carbohydrates were not present in the stem bark extract of *S. kunthianum* as shown in Table 4.1. The different phytochemical constituents of the stem bark extract of *S. kunthianum* plays a vital role in the development of drugs utilized for treating diseases in humans and animals. In the field of ethano-medicinal practice, *S.kunthianum* contains saponins which are among the secondary metabolites present in the extract. The *S.kunthianum* extract has shown various beneficial effects including anti-inflammatory properties which are useful in treating chronic inflammatory condition. The oral route was used to assess the 72-hour acute toxicity of the aqueous-methanol stem bark extract from Stereospermum kunthianum in diabetic rats. The extract induced no acute toxic manifestation or death of the adult Wistar rats even when given a dosage of up to 5000 mg/kg. According to the guidelines set by OECD, this level is considered practically non-toxic [27]. Similarly, Lorke showed that LD50 value greater than 1g (1000 mg/kg) for a test substance or chemical is considered as only slightly toxic (relatively safe) [17]. The assessment of the stem bark extract was beneficial in establishing the appropriate dosages for the experiment and assessing the safety level of the plant extract.

Diabetes is a chronic metabolic disorder associated with pain and inflammation caused by the body's resistance to insulin which is commonly associated with diabetes. In diabetes mellitus, glucose cannot be converted into energy due to lack of insulin or resistance of body cells to insulin or both. The acute complications of diabetes mellitus are hyperglycaemia, ketoacidosis, and hyperosmolar non-ketonic coma, while the long-term systemic complications include diabetic nephropathy, which may lead to amputation of joints, microangiopathy, diabetic neuropathy retinopathy, artherosclerosis, and infections [28,29]. Alloxan monohydrate is a toxic glucose analogue used to induce type II diabetes in laboratory test animals, where it selectively destroys insulin-producing cells in the pancreas stimulating the Type-II diabetes [30,31]. Inflammation is an important physiological mechanism for initiation of tissue repair; however excessive inflammatory reaction is deleterious to the body system. The signature of inflammation is the 'tetrad response' of dolor (pain), rubor (redness), calor (heat), and tumor swelling. The *S. kunthianum* extract has been known to have several pharmacological effects, including wound healing, anti-microbial, anti-diabetic and anti-inflammatory activities. The egg albumin-induced paw oedema is used to screen the anti-inflammatory activity of the stem bark extract of *S. kunthianum* in diabetic rats. The extract of *S. kunthianum* demonstrated anti-inflammatory effect using different inflammatory animal models. The egg albumin induced paw oedema was used in this study because it's the most commonly used anti-inflammatory model, less expensive and has simple procedure. In the present research, the saponins rich extract exerted the maximum paw oedema reduction at the 60_{th} minute due to inhibition of inflammatory mediators which caused a significant inhibition of paw edema in extract groups in comparison with negative group. The aqueous-methanol extract of S. kunthianum at (100, 200 and 400 mg/kg) produced a non-dose-dependent and the inhibition produced was less significant when compared to that of the standard drug (ASA 50 mg/kg). These findings corroborated results shown by Nwinyi *et al.* in normal rats [32]. The *S.kunthianum* extract has analgesic properties and can inhibit the action of prostaglandins through reduction of vascular permeability and fluid exudation, probably by preventing the contraction of endothelial cells, which causes the suppressing of oedema which is in agreement with the work done by Ching *et al* [13]. This effect leads to relief from acetic acid-induced pain. This analgesic activity is attributed to the presence of saponins, terpenes, and tannins in the extract, which also have anti-inflammatory effects [33-39]. At the end of the acetic-acid writhing test study, *S. kunthianum* at a dosage of 200 mg/kg demonstrated a greater percent inhibition of nociception compared to acetyl salicylic acid (ASA) at a dosage of 50 mg/kg administered through intraperitoneal injection, both having a similar percent inhibition of 96.8%. Nevertheless, the stem bark extract exhibited lower inhibition of pain perception when administered at doses of 100 and 400 mg/kg i.p compared to acetyl salicylic acid, and this effect was not dependent on the duration of administration. At 90, 120, and 180 minutes, intraperitoneal administration of stem bark extract of S. resulted in a dosage of 200 mg/kg. The analgesic effect of S. kunthianum was almost equal to that of ASA (50 mg/kg). ASA which is a standard non-steroidal anti-inflammatory drug also produced similar results, indicating that *S. kunthianum* has a similar inhibitory effect. The antinociceptive action of stem bark extract of S. kunthianum could be due to its action on visceral receptors which are reactive to acetic acid thereby preventing prostaglandin synthesis. Moreover, the outcome of the research achieved in the writhing test is comparable to the result of the egg-albumin paw oedema test. The findings from the research suggest that the stem bark extract of *S. kunthianum* shows promise as a potential analgesic agent that could offer similar effects as acetyl salicylic acid. This is because the extract effectively inhibited acute inflammation and the development of pain. The acetic-acid writhing test is effective but it's a non-specific pain model and many compounds which belong to diverse pharmacological categories like anti-inflammatory drugs, some non-analgesic drugs like clonidine and haloperidol, calcium channel blockers, opiods, anticholinergic, anti-histamines, corticosteroids also give positive results of analgesia using this model [34,35]. Because of lack of specificity of this model, positive results from the writhing test were confirmed using formalin test which is a more specific pain test [36-39]. The result obtained from this study is an indication that S. kunthianum stem bark extract has the potential of being developed into an analgesic agent with comparable effects as those of acetyl salicylic acid since the extract inhibited acute inflammation and pain development. This is a possible rationale for its use in traditional human medicine for pain relief. The formalin-induced pain model was further used to elucidate the mechanism of pain and analgesia. The nociceptive impact was recorded in two separate stages. In the early phase of the formalin test, the administration of stem bark

extract of *S. kunthianum* produced a lower pain inhibition. In the early phase of the formalin test in diabetic rats, the *S. kunthianum* extract at doses of 100 and 200 mg/kg showed minimal pain-relieving effects, whereas the pain-relieving effects of acetylsalicylic acid (ASA) at a dose of 50 mg/kg were high in the early stage. Acetylsalicylic acid is a known non-steroidal anti-inflammatory and antipyretic agent which is used to reduce pain and inflammation. The most potent amount of stem bark extract (400 mg/kg, i.p.) did not significantly reduce pain in the initial phase (0-15 minutes). The research on pain relief also indicated that S. In all the doses tested (100, 200, and 400 mg/kg), *S. kunthianum* showed a notable decrease in formalin-induced pain during the late phase (30-45 min), while ASA had a lesser analgesic impact at the same doses. However, the stem bark extract of *S.kunthianum* showed increased pain-relieving effects at the later stage of the pain test when given a dose of 400 mg/kg suggesting a possible prolongation of anti-nociception effect based on increased dose of extract. This nociceptive action is due to presence of saponins and tannins in the extract and these phytoconstituents have anti-inflammatory and nociceptive properties. The extract of S. kunthianum at doses of 100, 200, and 400 mg/kg displayed mild pain-relieving effects in both stages of the formalin-induced pain model. This indicates that the pain-relieving impact of the stem bark extract of *S. kunthianum* involves both neurogenic (centrally) and inflammatory (peripheral) mechanisms which occurs when the sensory nerve fiber is stimulated by formalin, leading to a decrease in the release of inflammatory substances like histamine, prostaglandins, and bradykinin [40]. However studies have shown that narcotics, which are centrally acting drugs, effectively hinder both phases equally. On the other hand, peripheral acting drugs like diclofenac and piroxicam specifically hinder the later stage. The findings are in alignment with previous research that demonstrated that the stem bark of *S. kunthianum* has been found to possess pain-relieving properties in normal rats, as observed in the studies conducted by Nwinyi et al and Ching et al [13,32]. There were certain limitations in this particular research. We only investigated the impact on behavior in diabetic rats that were given stem bark extract of *S. Kunthianum* reacts to pain by utilizing the pain models. More investigation is required to explore the specific mechanism of the extract from *S. kunthianum* and its role with the pain receptor [41].

21. Conclusion

These results suggests that the aqueous-methanol stem bark extract from *S. kunthianum* plant did not cause acute toxicity after administration of a single dose of 5000 mg/kg body weight of the rat. This extract was found to be relatively safe for the adult Wistar rats due to no deaths was recorded and no observable clinical signs of toxicity in the acute toxicity test. The extract's lethal dose (LD50) was determined to be ≥ 5000 mg/kg when administered orally, this shows *S. kunthianum* has no toxicity in adult Wistar rats at tested doses. This study has provided insight on the safety of aqueous methanol stem bark extract of *S. kunthianum*. Through this research, the phytochemical components present in the extracts which were saponins, terpenes, tannins, and steroids were identified. This shows that the isolated phytochemical components could be potential analgesic and anti-inflammatory agent. Using the animal models, the analgesic and anti-inflammatory properties of the extract was established. The dose (100 mg/kg) of the extract displayed the highest anti-inflammatory effect. The extract at 200 mg/kg had almost equal potency to acetylsalicylic acid (ASA, 50 mg/kg) in terms of reducing inflammation. The extract had weak pain inhibitory effects at both the early and late phases of the formalin test. In addition, these data provides evidence of antinociceptive effect of the stem bark extract of *S. kunthianum* which may be due to the presence of saponins and tannins which is probably related to inhibition of prostaglandin synthesis via inhibition of cyclooxygenases. The results corroborate the traditional use of S. kunthianum stem bark extract in the treatment of inflammation and pain-related health conditions. The results validated the ethno-medicinal use of *S. kunthianum* stem bark extracts in the management and treatment of pain and inflammation. The stem bark extract of *S. kunthianum* therefore has the potential to be developed as anti-inflammatory and analgesic agent in diabetic rats. This research is the first to report analgesic and anti-inflammatory properties of *S. kunthianum* in diabetic rats. However further studies are required to isolate and identify the active compound (s) from the plant, the extract should be evaluated for its effects on mediators of inflammation such as prostaglandins, leukotrienes, histamine, bradykinin, platelet activating factor and interleukin-1. A sub-acute and chronic toxicity studies should be conducted to determine the effects of long terms, low dose exposure to the stem bark extract of S. kunthianum and the mechanism of action of the extract on the measured parameters should be evaluated.

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