Neutralizing Effect of *Solanum dasyphyllum* Schumach.& Thonn Extract against*Naja nigricollis* Venom-induced Toxicity

ABSTRACT

Background: Clinical treatments for snakebite envenomation typically involve the administration of antivenom, which can lead to various side effects. However, due to the limitations of conventional antivenoms in effectively treating snakebite envenomation, scientists are now exploring alternative sources for potential antivenom compounds, particularly those derived from plants. *Solanum dasyphyllum*belongs to the *Solanaceae* family. This plant has been found to possess several beneficial properties, including neuromuscular, anti-poisoning, and antispasmodic effects.

Aim of the study: This study aimed to investigate the phytochemical constituents and the*in-vivo* detoxifying effect of *S. dasyphyllum* extract against *N. nigricollis*-induced toxicity.

Materials and Methods: Phytochemical screening and anticoagulant assays were conducted using standard procedures. The neutralizing and detoxifying effects of *S. dasyphyllum* were investigated *in-vivo* using locally bred adult Swiss mice.The neutralizing effect was evaluated in mice by administering a mixture of *N. nigricollis* venom and methanol leaf extract of *S. dasyphyllum*(100-400 mg/kg). The detoxifying effect was investigated by administering *S. dasyphyllum* via the intramuscular route 10 minutes after inoculation with *N. nigricollis* venom through the intraperitoneal route.

Results: The results of phytochemical screening revealed the presence of tannins, cardiac glycosides, saponins, flavonoids, and alkaloids, and the absence of anthraquinone and starch.The results of the clotting time of human blood treated with *N. nigricollis* venom showed a significant reduction in the clotting time with an increase in the concentration of methanol leaf extract of *S. dasyphyllum*. Additionally, the mortality of the mice that were pre-treated with *N. nigricollis* venom before injected with various doses of plant extract and those that were co-administered with the venom and plant extract significantly lower compared to mice

administered with the venom alone.*S. dasyphyllum* significantly reduced the extent of lipid peroxidation and the activity of the enzyme acetylcholinesterase compared to the untreated group.

Conclusions: *S. dasyphyllum* possesses antivenom activity against *N. nigricollis*, and this result further supports the ethnomedical use of the plant in the treatment of snakebite envenomation.

Keywords: Naja nigricollis, Solanum dasyphyllum, oxidative stress, Anticoagulant, antivenom, toxicity, Lethal dose.

INTRODUCTION

Snake envenomation is a neglected tropical disease that causes acute medical emergencies, especially in tropical regions (Gbolade, 2021). According to the World Health Organization, snakebites affect 5.5 million people annually, resulting in approximately 400,000 amputations and 120,000 deaths (World Health Organization, 2020). The population most at risk is predominantly young men engaged in agricultural or pastoral labour (Funmilola et al., 2020).The accurate assessment of the impact of snakebites in Sub-Saharan Africa is hindered by insufficient data. Nevertheless, recent estimates suggest that there are over 1,000,000 snakebites, resulting in 7,000-20,000 deaths and nearly 6,000 amputations annually in Sub-Saharan Africa and Nigeria (Abubakar et al., 2000). It is estimated that approximately 3,557 to 5,450 deaths due to snakebite envenomation occur in West Africa each year (Habib et al., 2015), and Nigeria is reported to account for a fifth of all snakebite cases in the West African region, representing 174 cases per 100,000 hospitalizations (Chippaux, 2011).

The venomous snakes in Africa belong to four main families: *Colubridae, Elapidae, Viperidae, and Hydrophidae*.According to Warrell (1987), three snake species: carpet viper (*Echis ocellatus*), black-necked spitting cobra (*N. nigricollis*), and puff adder (*Bitis arietans*) belonging to the *Viperidae* and *Elapidae* families are the most significant snakes related to envenoming in Nigeria (Abubakar et al., 2010). The majority of snakebite cases in Nigeria are caused by carpet vipers, followed by cases from *N. nigricollis* (Adzu et al., 2005). The envenomation of these

snakes can result in neurotoxicity, severe local symptoms such as swelling, blistering, and necrosis, as well as general systemic symptoms of envenomation (Sharma et al., 2004).

Venom is a mixture of enzymes that includes small peptides, metal ions, amines, and carbohydrates.It exerts neurotoxic and cytotoxic effects on the snake's prey and is responsible for various pharmacological reactions such as cell destruction, haemolysis, paralysis, necrosis, haemorrhage etc. (Meyer et al., 1997). Anti-venom immunotherapy is the only treatment available for snake envenomations (Morais and Massaldi, 2008). However, it is associated with many side effects, including anaphylactic shock, pyrogen reaction, and serum sickness (Williams et al., 2010). In addition to the side effects, sub-Saharan Africa faces challenges related to the quality, quantity, specificity, access, and distribution of antivenoms, which significantly increase the burden of morbidity and mortality (Harrison et al., 2009). In this context, researchers have been exploring natural products and plant extracts to investigate their antivenom activity, as well as their anti-myotoxic, anti-hemorrhagic, and anti-inflammatory properties (Funmilola et al., 2020b).

Solanum dasyphyllum, whose name has been verified on www.worldfloraonline.org, belongs to the *Solanaceae* family (Obade et al., 2018). It is an erect, perennial herb with stems that are often woody, growing 50-100 cm tall. The plant is branched at the base, with branches heavily armed with 2-7 mm long prickles.*S. dasyphyllum* is used ethnomedically to treat poisoning (Oyinloye et al., 2020). The plant is reported to possess anticonvulsant, antioxidant, and neuromuscular properties (Adesina, 1983). In the southwestern part of Nigeria, the fruit of *S. dasyphyllum* is commonly mixed with local black soap and applied to incisions at snakebite sites. This practice is believed to help remove venom from the bite site and reduce its absorption into the bloodstream. However, the potential of *S. dasyphyllum* to treat snakebites has not been scientifically evaluated. Therefore, this study aims to conduct an in vivo assessment of the anti-snake venom-neutralizing properties of *S.dasyphyllum* methanol leaf extract.

MATERIALS AND METHODS

Plant Collection and Preparation

The leaves of *S. dasyphyllum* were collected from Odeomu town (Latitude: 7°32'0" North; Longitude: 4°24'0" East) in Ayedaade Local Government Area, Osun State. They were identified and authenticated in the Department of Botany at Obafemi Awolowo University, Ile-Ife, Osun State, and a voucher specimen number IFE-17489 was issued. The leaves were cleaned, dried, and pulverized into powder. The powdered leaf (500 g) was soaked in 250 mL of 85% methanol for 72 hours with constant stirring. The extract was filtered successively using muslin cloth and Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator and then lyophilized to dryness.

Venom Procurement

Lyophilized *Naja nigricollis* venom was purchased from the Department of Pharmacognosy and Drug Development at Ahmadu Bello University, Zaria, Nigeria, and stored at 4°C. Before use, the venom was reconstituted in a phosphate buffer at pH 7.2, centrifuged at 2000 rpm for 10 minutes, and the resulting supernatant was used for further studies.

Anti-snake venom (ASV)

The polyvalent antivenom serum (ASV) was purchased from the local medical store. It was produced by Vins Bioproducts Limited in Hyderabad, India.

Experimental Animals

Locally bred adult Swiss mice, weighing 20 ± 5g of both sexes, were used for this research. The mice were purchased from the Faculty of Veterinary Medicine, University of Maiduguri. The animals were fed standard mouse pellets, and water was supplied ad libitum. They were allowed to acclimatize before the tests. The animals were handled in compliance with national regulations for animal research.

Phytochemical screening

The methanol extract of *S. dasyphyllum* was assessed for the presence of phytochemicals following the method outlined by Sofowora (1993) to confirm the existence of secondary metabolites such as alkaloids, tannins, saponins, carbohydrates, flavonoids, terpenes, glycosides, and cardiac glycosides.

Neutralization of Anticoagulant Activity in Crude Extract

The plasma coagulation property was determined according to the method outlined by Condrea et al. (1981). Human citrated plasma (200 µL) was incubated with 10 µL of diluted venom at 37°C, against a light source; the clotting time was recorded after the addition of 25 mM calcium chloride. Human plasma incubated with PBS alone served as the control. For inhibition studies, 0.2 mL of 1.0 mg venom in 1 mL PBS was pre-incubated with various concentrations of methanol leaf extract of *S. dasyphyllum* (0.2 mL of 200, 400, 600, 800µg/mL) at 37°C for 45 minutes. Clotting time was recorded after the addition of calcium chloride.

Lethal Toxicity Neutralization of *Naja nigricollis* Venom by *Solanum dasyphyllum* Extract

The study utilized a median lethal dose (LD_{99}) of 7.57 mg/kg of *N. nigricollis* venom administered via intraperitoneal injection (i.p), as previously reported by Funmilola et al. (2021). The effect of *S. dasyphyllum* extract on the LD_{99} of venom was determined by grouping the albino mice into six groups of five animals (n=5); group 1 (control) received normal saline, group 2 (positive control) received LD_{99} venom only, while group 3 (Negative control) received polyvalent antivenom. Group 4,5 and 6 (serving as treatment groups), were pre-treated with i.p LD_{99} of the venom, followed by an i.m injection of 100mg, 200mg and 400mg *S. dasyphyllum* extract 10 minutes later.Mortality rates were recorded over a 24-hour period.

Detoxification of *Naja nigricollis* Venom by *Solanum dasyphyllum* Extract

The lethal toxicity neutralization effect of *S. dasyphyllum* extract on the LD_{99} of venom was determined by grouping the albino mice into five groups of five animals (n=5); group 1(Negative control)was given normal saline, while Group 2 (Positive control) was given i.p. dose of LD_{99} venom only. Groups 3,4 and 5 (serving as the treatment group) were injected (i.p) with pre-incubated mixture of venom LD_{99} with 100mg, 200mg and 400mg of the extract at 37^0C for 10

minutes. Behavioural changes and mortality were observed every hour for up to 24 hours. The extent of lipid peroxidation and acetylcholinesterase activity was assessed in the brain of both the deceased and surviving animals. The brain was homogenized in phosphate buffer pH 7.4 and centrifuged at 3000rpm. Brain homogenate was used to assay acetylcholinesterase activity and lipid peroxidation.

Lipid Peroxidation Inhibitory Activity

Using the method described by Ohkawa et al. (1979), the extent of lipid peroxidation was determined by measuring the formation of Thiobarbituric acid reactive substances (TBARS). 0.4 mL of the brain homogenate was mixed with 1.6 mL of 0.15M Tris-KCl buffer, to which 0.5 mL of 30% TCA was added. Then, 0.5 mL of 0.75% TBA was added and placed in a water bath for 45 minutes at 80°C. The solution was then cooled on ice and centrifuged at 3000 g. The clear supernatant was collected, and the absorbance was measured against a reference blank of distilled water at 532 nm. The MDA level was calculated according to the method of Adám-Vizi and Seregi (1982). Lipid peroxidation was calculated in units/mg protein using a molar extinction coefficient of 1.56 x 10^5 $M^{-1}Cm^{-1}$.

Assessment of Acetylcholinesterase (AChE) Activity

AChE activity was measured using the spectrophotometric method developed by Ellman et al. in 1961. 0.1 mL of 0.01 M DTNB was added to 2.6 mL of 0.1 M phosphate buffer (pH 8.0). Then, 0.04 mL of brain homogenate was added to the mixture, followed by incubation for 5 minutes. After incubation, 0.04 mL of substrate (0.075 M acetylcholine iodide) was added to the reaction mixture. Absorbance readings were taken at 420 nm continuously for 3 minutes at 30-second intervals. The results were expressed in µmol/min/mg protein using a molar extinction coefficient of 1.36 x $10^{-4}M^{-1}cm^{-1}$.

Statistical Analysis

The experimental data were analyzed using SPSS 20.0 software, and the results were expressed as mean ± SD.All data were evaluated using analysis of variance (one-way ANOVA), and a P-value of <0.005 indicates that the difference was statistically significant.

RESULTS

The results of the phytochemical screening of the crude methanol leaf extract of *S. dasyphyllum*, presented in Table 1, show the presence of carbohydrates, saponins, flavonoids, terpenoids, cardiac glycosides, tannins, steroids, glycosides, and alkaloids. Anthraquinone and starch were not detected. Figure 1 presents the results of the neutralization of *S. dasyphyllum* against the anticoagulant activity of *N. nigricollis* venom, as measured by the time taken for the blood to clot.The clotting time was reduced in a concentration-dependent manner by the various concentrations of the extract of *S. dasyphyllum*. The results of the neutralization effect of different doses of *S. dasyphyllum* extract in mice pretreated with *N. nigricollis*venom LD_{99}, as shown in Table 2, indicate that *S. dasyphyllum* can mitigate the lethal effect of *N. nigricollis* venom. The dose of 100 mg/kg exhibited the highest detoxifying potential, followed by 200 mg/kg. Table 3 presents the results of the detoxifying effect of different doses of methanol leaf extract of *S. dasyphyllum*on *N. nigricollis* venom. There was a significant increase in the survival rate of the animals in the treatment groups compared to Group 2 (positive control). Figure 2 illustrates the beneficial impact of various doses of *S. dasyphyllum* methanol leaf extract on acetylcholinesterase activity and lipid peroxidation in the brains of mice induced with *N. nigricollis* venom. The activity of acetylcholinesterase in the brain increases with a higher dose of the extract. The treatment group that received a dose of 100 mg/kg exhibited lower acetylcholinesterase activity compared to the other treatment groups that received 200 mg/kg and 400 mg/kg. Additionally, lipid peroxidation increases with a higher dose of the extract. The level of lipid peroxidation in the treatment groups (200 mg/kg and 400 mg/kg) was significantly higher than that in the control group. However, the MDA content in the control group and the treatment group (100 mg/kg) is relatively similar. Hence, the optimal dose of the extract is 100 mg/kg.

Table 1: Phytochemical Constituents of Methanol Leaf Extract of *Solanum dasyphyllum*

Phytochemical	Observation
Alkaloids	+
Flavonoids	+
Tannins	+
Terpenoids	+
Steroids	+
Glycosides	+
Anthraquinone	–
Cardiac glycoside	+
Sugar	+
Starch	–
Saponins	+

Key: + = Present

- = Absent

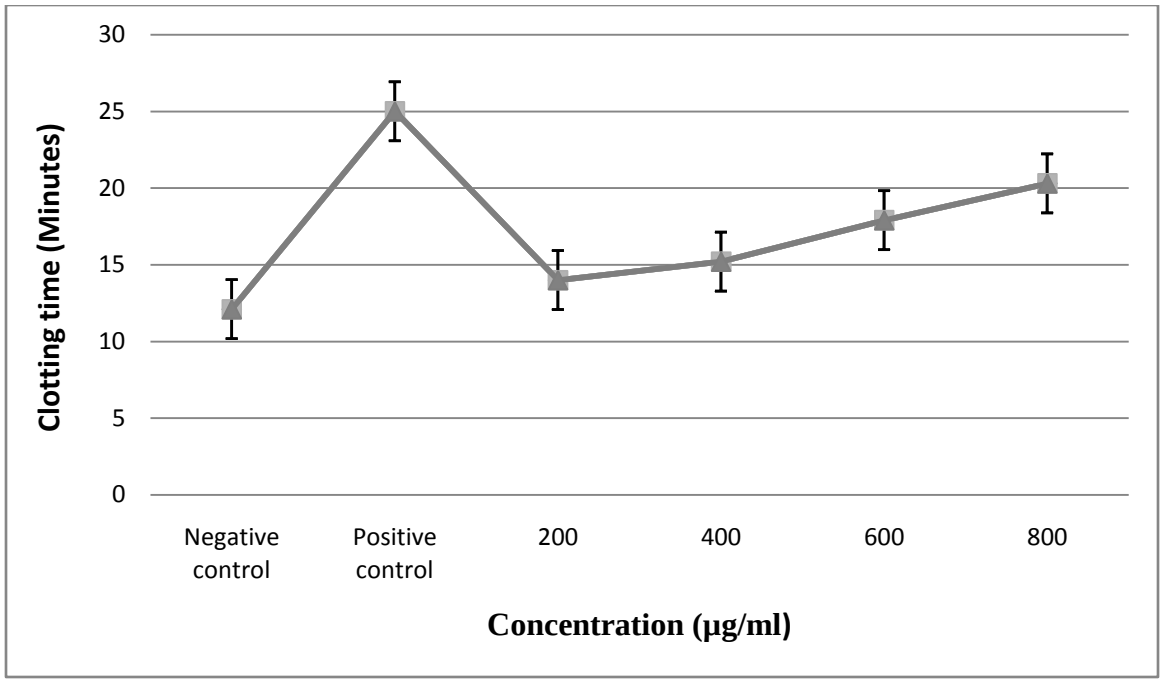


Figure 1: Effect ofMethanol leaf extract of *S. dasyphyllum*on *N. nigricollis* venom anticoagulant activity

Table 2: Lethal Toxicity Neutralization of *Naja nigricollis* Venom by *Solanum dasyphyllum* Extract

Group	Concentration	Number of survival/ total number of animals	% mortality
1	Control (Phosphate buffer only)	5/5	0
2	Positive control (LD_{99} Venom only)	0/5	100
3	Negative control (LD_{99} + Antivenom)	4/5	20
4	LD_{99} +100mg/kg of extract	4/5	20
5	LD_{99} + 200mg/kg of extract	3/5	40

6	LD_{99} + 400mg/kg of extract	1/5	80

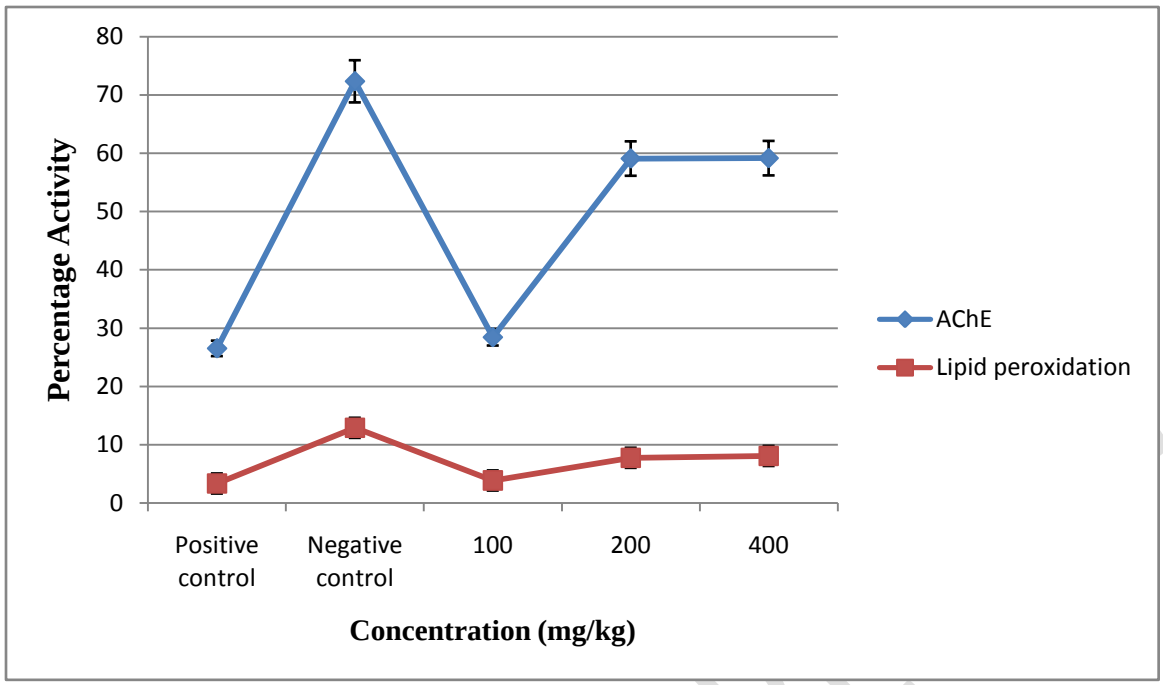
Table 3: Detoxification of *Naja nigricollis* Venom by *Solanum dasyphyllum* Extract

Group	**Extract Dose**	**Number of survival/ total number of animals**	**Average death time (hour)**	**% mortality**
Group 1 (+ve control)	Normal saline only	6/6	0	0
Group 2 (-ve control)	LD_{99}only	0/6	1.45±1.02	100
Group 3	LD_{99} +100mg/kg	6/6	0	0
Group 4	LD_{99} +200mg/kg	6/6	0	0
Group 5	LD_{99} +400mg/kg	5/6	0.8	16.6

Key:

+ve: Positive

-ve: negative



Key: AChE: Acetylcholinesterase

Figure 2: Evaluation of acetylcholinesterase (AChE) activity and extend of lipid peroxidation in venom-induce toxicity in mice

DISSUSION

The application of herbal remedies in the treatment of various diseases is growing at a faster rate globally; the low toxicity and easy accessibility are some of the major contributors to this global acceptance. According to Gbolade(2021), snakebite envenomation is one of the leading causes of death in Nigeria, particularly in rural areas with limited access to medical care. Like plants, snake venom is a complex mixture of biologically active compounds capable of exhibiting various pharmacological actions such as neuromuscular blockage, haemorrhage etc (WHO, 2014). However, the most effective and acceptable therapy for snakebite victims is the immediate administration of anti-venom following envenomation (Mahanta and Mukkerjee, 2001). The use of plants in the management of snakebite envenomation has been widely accepted and recognized (Alam and Gome, 2003), therefore, the search for novel natural or synthetic molecules capable of inhibiting the local damage caused by snakebites envenomation is a promising field of research.

The phytochemical screening of methanol leaf extract of *S. dasyphyllum* revealed the presence of alkaloids, tannins, flavonoids, terpenoids, cardiac glycosides, and sugars, but it was devoid of starch and free anthraquinone. The result of the crude extract of *S, dasyphyllum* correlated with those previously reported by Sodeinde*et al*., (2019), who worked on *S. dasyphyllum* and *S. incanum L*, another species of the *Solanaceae* family.

The anticoagulant in the venom directly degenerates fibrinogen (factor 1), which in turn reduces the formation of fibrin which forms the initial clot. The neutralization of the anticoagulant activity of *N. nigricollis* venom by *S.dasyphyllum* extract was demonstrated by the ability of the plant extract to inhibit fibrinogen breakdown caused by snake venom, probably arresting excessive bleeding caused by snake envenomation. The clotting time of *N.nigricollis* venom reduces significantly with an increase in the concentration of methanol leaf extract of *S. dasyphyllum*. This implies that *S. dasyphyllum* methanol leaf extract has the potential to arrest bleeding caused by fibrinogen degradation.(Lugun et al., 2022)reported a similar result about the antithrombotic activity of *Solanum xanthocarpum*, a *Solanaceae* family, therebyinhibiting thrombin-induced platelet activation.

In this study, 2 different *in-vivo* approaches were used for assessing the antivenom potential of *S. dasyphyllum*: neutralizingeffect of various doses of *S. dasyphyllum* methanol leaf extract (i.m) on albino mice 10 minutes after injected with *N. nigricollis* venom (i.p) and detoxification of the lethal venom effect of *N. nigricollis* by methanol leaf extract of *S. dasyphyllum* using Alam and Gome's method and. Mice treated with the LD_{99} showed excitement, followed by restlessness, paralysis and death; hence, the mice exhibited a classical symptom of neurotoxicity. Although, the component of the plant extract responsible for the antivenom activity observed in the present study has not yet been identified, the *N. nigricollis* venom neutralization and detoxification ability of *S. dasyphyllum* assessed by the*in-vivo* lethality assay in mice confirmed the antisnake venom potential of the extract since it was able to reduce the mortality due to envenomation. These activities may be ascribed to the phytochemicals such as flavonoids, saponins, tannins, alkaloids, and terpenoids present within them.Mors *et al*., (2000) reported that phenolic compounds, saponins, flavonoids and tannins can bind to proteins and can directly act on venom constituents. Similarly, Lans *et al*., (2001) reported that plant alkaloids are effective against snakebites. Furthermore, cardiac glycosides are known to act as Na^+/K^+ pump inhibitors. This

causes an increase in the level of sodium ions in the myocytes, which leads to a rise in the level of calcium ions which may be important in counteracting the hemorrhagic effects

All vertebrates contain acetylcholinesterase (AChE), which is especially abundant in the muscles and nervous systems(Tougu, 2005). The primary function of the enzyme, which is present in both synaptic and non-synaptic tissues, is to hydrolyze the neurotransmitter acetylcholine (ACh), which is necessary for regular cholinergic transmission and optimal neuromuscular function (Herlenius and Lagercrantz, 2001). With the exception of mambas, all Elapidae venom contains acetylcholinesterase. Compared to human acetylcholinesterase, the venom acetylcholinesterase has a higher affinity for acetylcholine. As a result, it binds to acetylcholine molecules in the brain and inhibits human AChE from degrading them(Cousin et al., 1996). This eventually causes an accumulation of Ach in the synaptic gap, which causes excessive neuronal stimulation and prolonged nerve impulse transmission. The disruptions of the cholinergic transmission canresults into muscle spasms, paralysis, respiratory distress, seizures and cognitive impairment. The findings from this study revealed that *S. dasyphyllum* extract was able to significantly lower the activity of *N. nigricollis* venom acetylcholinesterase. This finding was concise with what reported by (Obade et al., 2018) and (Houghton and Howes, 2017)that *S. dasyphyllum* possesses anticholinesterase activity; thus, they are potent cholinergic blocker.

The production of reactive oxygen species (ROS), which can cause oxidative stress and encourage lipid peroxidation(Bochkov et al., 2010), is another important mechanism of venom envenomation due to cell membrane destabilization. Venom is a mixture of pharmacologically active molecules, such as enzymes, peptides, nucleotides etc that can damage cellular components such as lipids, proteins and DNA. Venom-induced lipid peroxidation has the potential to harm cell membranes, impairing cellular functions and causing dysfunction. Since brain membrane lipids are highly susceptible to oxidation due to their high polyunsaturated fatty acid content, lipid peroxidation (LPO) is a crucial indicator of oxidative stress.*S. dasyphyllum* has been shown to have antioxidant properties in earlier research. In the current investigation, LPO in the mice's brains that were given *N. nigricollis* venom was inhibited by *S. dasyphyllum* extract in a dose-dependent pattern. According to (Adesina, 1983), the leaves of *S. dasyphyllum* contain a variety of phytochemical constituents, including alkaloids and flavones, which may indicate the plant's antioxidant capabilities.

CONCLUSION

The present study demonstrates the mitigation of *N. nigricollis* induced toxicity by *S. dasyphyllum* extract possibly via detoxification, neutralization and antioxidant mechanisms. The findings from this study suggest that the *S. dasyphyllum* may be a promising candidate for the management of *N. nigricollis* snake envenomation.

ETHICAL APPROVAL

The study was approved by the Animal Research Ethics Committee, University of Maiduguri, FP/022020/PGb01.

ABBREVIATION

DTNB	5,5-dithiobis-(2-nitrobenzoic acid)
AChE	Acetylcholinesterase
MDA	Malondialdehyde
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TBARS	Thiobarbituric acid reactive substance
I.p	Intraperitoneal injection
MLD	Minimum lethal dose
S. dasyphyllum	*Solanum dasyphyllum*Schumach. & Thonn
N. nigricollis	*Naja nigricollis*

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