



PRELIMINARY PHYTOCHEMICAL SCREENING, TOXICOLOGICAL AND ANTIVENIN PROPERTY OF THE STEM BARK OF *NEOCARYA MACROPHYLLA* ON *NAJA NIGRICOLIS* VENOM

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ABSTRACT

Neocarya macrophylla is a West African plant species often employed in ethnomedicine for the treatment of pain, inflammation, skin infections, asthma and snakebite. The stem-bark of the plant was pulverized and extracted with methanol by maceration. Preliminary phytochemical screening of the crude methanol extract revealed the presence of secondary metabolites including carbohydrates, alkaloids, flavonoids, anthraquinones, saponins, tannins, glycosides, steroids and triterpenes. Acute toxicity studies conducted in mice (*i.p*) using Lorke's method gave an LD₅₀ of 283 mg/kg, suggesting the plant to be relatively toxic. The antivenin effect of the crude methanol extract was tested against cobra (*Naja nigricolis*) venom using laboratory animals. Maximum protection was observed at an administered dose of 40 mg/kg with 100% survival, 83% at 20 mg/kg and relatively lower survival rate (67%) at a dose of 2 mg/kg and 0% at 5 mg/kg. The results suggest that the crude methanol extract of *Neocarya macrophylla* contains bioactive constituents with significant antivenom activity both *in vitro* and *in vivo* and lends credence to traditional use of the plant in the management of snakebite.

KEYWORDS: *Neocarya macrophylla*, Phytochemical screening, Antivenin, *Naja nigricolis*.

INTRODUCTION

Majority of the rural populations in Africa and other developing countries depend on traditional medicines for prevention of illnesses or maintenance of well-being. This is due to its relatively easy access, affordability and wider acceptable to most populations [1]. The World Health Organization estimates that about 80% of the world population relies on herbs which contain some bioactive organic secondary metabolites such as tannins, alkaloids, flavonoids, terpenoids and steroids [2, 3]. These compounds produce definite physiological action on human body.

Envenomation resulting from snakebite is a public health problem in many developing countries such as Nigeria [4]. The annual snakebite incidences in savannah region of Northern Nigeria have been estimated to be 497 per 100,000 populations with 12.2% mortality [10]. The world prevalence is more than 3million with 150,000 deaths, annually [24]. The current available therapy for snakebite victims is the immediate administration of antivenin but, the

supplies of the drug is severely restricted and largely unavailable due to access and cost [5, 6]. The antivenin often precipitates allergic reactions in several patients, thereby limiting usage in those persons [7]. Furthermore, the cold storage requirements for the drug limits distribution especially in developing countries such as Nigeria with limited power supply [8]. All these drawbacks compel the search for effective alternative antidotes that are less expensive, easily accessible and readily available.

The use of plants in the management of snakebite has long been recognized [6]. More scientific attention has been given to plant-derived antivenin since the last 20 years [7]. *Neocarya macrophylla* (Sabine) Prance (Chrysobalanaceae), formerly *Parinari macrophylla* Sabine is a shrub or small tree found in tropical and sub-tropical regions [9]. In Northern Nigeria, the plant is used in folkloric medicine to treat asthma, skin infections, wounds, dysentery, inflammations, pulmonary troubles and

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ear and eye infections [11]. The fruit is mostly eaten fresh or boiled with cereal to treat diarrhoea [12]. The nuts are usually roasted and enjoyed like cashews or almonds [13]. The plant is also employed to treat snakebite, pain and inflammations [Personal communication]; traditional healers usually boil the leaves or the stem bark in water and serve the snakebite victim. This work was carried out to validate the ethnomedicinal use of the plant as snake venom antidote.

Materials and methods

Collection and Identification of Plant material

The plant sample of *Neocarya macrophylla* was collected in November 2012 at Jega, Jega Local Government Area of Kebbi State. It was identified by U.S Gallah of the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University by comparing with herbarium reference voucher specimen (No. 3197).

Preparation of the extract

The stem bark was shade dried, pulverized, labelled and stored at room temperature for use. The powdered stem bark (3000 g) was extracted with methanol using maceration method for 3 days (three times), with occasional shaking. The extract was evaporated in-vacuo using rotary evaporator to afford a reddish-brown residue (396g) subsequently referred to as the crude methanol extract (ME).

Preliminary Phytochemical Investigation

Portion of the methanol extract was subjected to preliminary phytochemical screening for the presence of secondary metabolites in accordance with procedures as described by African Pharmacopoeia [14].

Venom sample

The venom of *Naja nigricolis* with LD₅₀ (9.55 mg/kg) was obtained from Prof. M.S. Abubakar, Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria.

Experimental Animals

Swiss albino mice of either sex (15-38 g) obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria, were used for the study. They were fed with laboratory diet and water *ad libitum* and maintained under standard conditions (12 h light and 12 h dark cycle) in propylene cages at room temperature.

LD₅₀ Determination

The method described by Lorke was employed. The route of administration was intra-peritoneal. In the first phase, nine mice of either sex were divided into three groups containing three mice each. The first,

second and third groups received 10 mg/kg, 100 mg/kg and 1000 mg/kg respectively. In the second phase, four animals were used. Each of the four animals received different doses of the extract which were: 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg [15].

In vitro snake venom detoxifying effect of the methanolic extract

Twenty four mice were divided into four groups (n=6). Group 1 (control) received 10 mg/kg of the venom only. Groups 2, 3 and 4 (treatment groups) received an equivalent of 10 mg/kg of the venom containing 20, 40 and 80 mg/kg of the extract respectively. The venom and the extract were mixed and incubated together at 37°C for 10 min prior to injection. The incubated mixture was then administered *i.p* to each mouse in the treatment groups. The animals were observed for mortality for 24 h [16].

In vivo snake venom detoxifying effect of the methanolic extract

Thirty mice were divided into five groups (n=6). Group 1 (control) received 10 mg/kg of the venom only. Groups 2, 3, 4 and 5 (test groups) received 2, 5, 10 and 20 mg/kg of the extract respectively (*i.p*). The test groups were then injected *i.p* with 10 mg/kg of the venom 30 min after injecting the extract. All animals were observed for mortality for 24 h [17].

Results and Discussion

The results of preliminary phytochemical screening, *in vitro* and *in vivo* detoxifying effect in mice of methanol extract of stem bark of *N. macrophylla* are presented in Tables 1, 2 and 3, respectively.

Preliminary phytochemical screening of the methanolic extract of stem bark of *N. macrophylla* revealed the presence of carbohydrates, alkaloids, flavonoids, anthraquinones, saponins, tannins, glycosides, steroids and triterpenes [Table 1]. These constituents have been reported to be associated with different pharmacological activities of plants [21].

The intraperitoneal LD₅₀ of the methanolic extract in mice was found to be 283 mg/kg. This suggests that the plant is relatively toxic [15].

The extract exhibited a significant *invitro* activity against the *Naja nigricolis* venom in mice. The maximum protective effect of the extract was observed at 40 mg/kg with 100% survival. The results of the study suggest that the extract might act by neutralising the activity of the venom at the site of bite, thereby reducing harshness of toxic

effects [18]. The result of the *in vivo* studies revealed a significant activity against *Naja nigricolis* venom in mice with 67% survival at 2 mg/kg. The mechanism by which the extract achieves its effect remains unclear, but the activity may probably be linked to the existence of active components/compounds present in the plant. The activity observed by the mice administered with extract may be attributed to the presence of any of the constituents such as alkaloids, flavonoids, tannins, saponins, steroids present in the plant extract. Flavonoids, polyphenols and terpenoids are reported to possess protein binding and enzyme inhibiting properties [19, 20]. Tannins are known to unspecifically inactivate proteins [16]. Triterpenoids may be involved in venom inactivation processes [20]; pentacyclic triterpenes (free or as glycosides) are found widely in several anti-snake venom plants and provide nearly 20% protection against snake venom [21]. Beta-sitosterol and stigmasterol have

been reported to play an important role in neutralizing snake venom-induced actions with nearly 70% and 80% protection, respectively [22]. Stigmasterol was recently isolated from the stem bark of *N. macrophylla* [23]. The observed activity of the extract might be linked to the presence of stigmasterol and other bioactive constituents present in the plant.

CONCLUSION

Neocarya macrophylla has demonstrated significant antivenin activity rationalizing its ethnomedicinal use in the treatment of snakebite.

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Table 1: Preliminary Phytochemical Screening of *N. macrophylla*

Constituents	Test	Inferences
Carbohydrates	a. Molisch's test	+
Glycosides	b. Fehling's test	+
Alkaloids	a. Mayer's test	+
	b. Dragendorf's test	+
Flavonoids	a. Ferric chloride test	+
	b. NaOH test	+
Anthraquinones	a. Borntrager's test	+
Saponins	a. Frothing test	+
Steroids & Terpenes	a. Lieberman-Buchard	+
	b. Salkowski test	+
Tannins	a. Lead Sub-acetate	+

Key: + presence of constituent - absence of constituent

Table 2: *In-vitro* detoxifying effect of the methanolic extract of *N. macrophylla* on *Naja nigricolis* venom

Group	Treatment	%Survival (within 24 h)
1	LD ₉₉	0%
2	LD ₉₉ + 20 mg/kg	83%
3	LD ₉₉ + 40 mg/kg	100%
4	LD ₉₉ + 80 mg/kg	33%

Table 3: *In vivo* detoxifying effect of the methanolic extract of *N. macrophylla* on *Naja nigricolis* venom

Group	Treatment	%Survival (within 24 h)
1	LD ₉₉	0%
2	LD ₉₉ , 2 mg/kg	67%
3	LD ₉₉ , 5 mg/kg	0%
4	LD ₉₉ , 10 mg/kg	0%
5	LD ₉₉ , 20 mg/kg	0%

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