TPT Trends in Pharmacology and Toxicology

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Antivenom Potential of *Catunaregam nilotica* Root-Bark Extract Against *Echis ocellatus* Venom Toxicity in Albino Rats

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ABSTRACT

Background and Objective: Snakebite envenomation caused by *Echis ocellatus* (West African carpet viper) remains a major public health issue in Sub-Saharan Africa, contributing significantly to morbidity and mortality. The envenomation commonly results in systemic hemorrhage, coagulation disorders, local tissue necrosis and multiple organ dysfunctions. This study, therefore, investigated the antivenom efficacy of the n-hexane fraction (n-HF) derived from the root-bark of *Catunaregam nilotica* against *E. ocellatus* venom-induced pathophysiological alterations in albino rats. **Materials and Methods:** Acute oral toxicity of the crude methanol extract of *C. nilotica* root bark was evaluated using Lorke's method, while the Median Lethal Dose (LD_{50}) of *E. ocellatus* venom was determined via intraperitoneal administration. Envenomed rats received treatments with the crude extract and its solvent fractions. Standard biochemical procedures were used to determine hepatic and renal function indices, hemostatic parameters (bleeding and clotting times, plasma fibrinogen levels), and lesion diameters. Data were analyzed using one-way ANOVA followed by Tukey's *post hoc* test ($p<0.05$). **Results:** The crude extract was found to be safe up to 5000 mg/kg body weight, indicating a wide margin of safety. Envenomed rats showed severe pathological effects, including hemorrhage, necrosis, coagulopathy, hepatic and renal impairment, and hemolysis. Administration of both the crude extract and n-HF significantly ($p<0.05$) improved survival, normalized liver and kidney biomarkers, shortened bleeding and clotting times, reduced hemorrhagic and necrotic lesions, and markedly inhibited hemolysis. **Conclusion:** The n-hexane fraction of *Catunaregam nilotica* root bark exhibited potent antivenom activity, effectively mitigating both systemic and local toxic effects of *E. ocellatus* venom. These findings provide scientific support for the potential development of *C. nilotica*-based phytotherapeutics as complementary or alternative antivenom agents.

KEYWORDS

Echis ocellatus, *Catunaregam nilotica*, antivenom, toxicity, coagulopathy, phytotherapy

ISSN: 2789-9446 (Online)
ISSN: 2789-9438 (Print)
https://doi.org/10.21124/tpt.2026.13.22

Received: 25 Oct. 2025
Accepted: 23 Jan. 2026
Published: 31 Mar. 2026

INTRODUCTION

Snakebite envenomation remains a serious but often overlooked public health issue. It primarily affects rural communities in developing countries, where access to healthcare and effective disease surveillance remains limited. The World Health Organization (WHO) classifies snakebite as a neglected tropical disease because of its high rates of death, disability, and economic loss among farming populations who are most at risk[1]. Globally, around 5.4 mL snakebites occur each year, resulting in about 2.7 mL cases of envenomation and between 81,000 and 138,000 deaths[2]. Many of those who survive are left with long-term disabilities that affect their livelihoods and quality of life. In Nigeria, *Echis ocellatus* accounts for more than 90 percent of snakebite deaths, particularly in the northern agricultural belt where most victims are small-scale farmers[3]. The venom of *E. ocellatus* is a potent mixture of toxins. Such as Snake-Venom Metalloproteinases (SVMPs), Phospholipase A_2 (PLA_2), and serine proteases interfere with blood clotting, destroy tissue structures, and can trigger massive bleeding or multi-organ failure[4]. The standard treatment Anti-Snake Venom (ASV), can neutralize circulating toxins but comes with several challenges. It is expensive, requires cold-chain storage, and is often scarce in rural health facilities. In addition, some patients experience severe allergic reactions, including serum sickness and anaphylaxis[5]. Unfortunately, ASV also does little to prevent the local tissue damage and necrosis that cause permanent disability among many survivors[6]. Because of these limitations, there is growing interest in safer, cheaper, and locally available therapeutic options.

Medicinal plants offer a promising alternative since many contain natural compounds with antioxidant, anti-inflammatory, and enzyme-inhibitory properties. *Catunaregam nilotica* (Rubiaceae); a shrub widely found in the dry regions of Africa. Traditional healers have long used it to treat snakebites, fever, and various infections[7]. Phytochemical studies of its root bark have identified several bioactive constituents, including flavonoids, alkaloids, tannins, and triterpenoids, all of which have known pharmacological potential[8]. Although ethnobotanical reports and preliminary laboratory findings suggest that *C. nilotica* may possess antivenom activity, comprehensive scientific validation is still limited. Therefore, this study investigates the *in vivo* and *in vitro* antivenom efficacy of *Catunaregam nilotica* root-bark extract and its solvent fractions against *Echis ocellatus* venom, with the goal of identifying plant-based therapeutic candidates that could improve snakebite management in resource-limited settings.

MATERIALS AND METHODS

Plant material collection and identification: Root bark of *Catunaregam nilotica* was collected in February, 2024 from Gabake Village, Zamfara State, Nigeria. The plant was authenticated by a Taxonomist, and a voucher specimen (KSUSTA/PSB/H/234A) was deposited at the Herbarium of Abdullahi Fodio (Kebbi State) University of Science and Technology, Aliero, Nigeria.

Preparation and fractionation of extract: The air-dried powdered root-bark (500 g) was soaked in 2.5 L of methanol for 72 hrs with intermittent shaking. The filtrate was concentrated at 40°C under reduced pressure to obtain the crude methanol extract of *Catunaregam nilotica* root bark (CMECNR). The extract was dissolved in water and successively partitioned with n-hexane, ethyl acetate, and n-butanol. Each fraction was dried and stored at 4°C until use[9].

Venom source and preparation: Lyophilized *Echis ocellatus* venom was obtained from Ahmadu Bello University, Zaria, in March 2024. It was dissolved in phosphate-buffered saline (PBS, pH 7.4), aliquoted, and stored at -20°C. Fresh dilutions were prepared for each test.

Experimental animals: Healthy male albino rats (180-220 g) were obtained from the KSUSTA animal facility and maintained under standard laboratory conditions with unrestricted access to feed and water. All procedures followed institutional ethical guidelines for animal care and use.

Acute oral toxicity: The acute oral toxicity of CMECNR was assessed using Lorke's method with doses ranging from 10 to 5000 mg/kg. Animals were observed for 14 days for behavioral changes or mortality[10]. The LD_{50} was calculated as the geometric mean of the highest non-lethal and lowest lethal doses using the formula:

$$LD_{50} = \sqrt{a} \times b$$

Where:

a = The highest dose at which no death occurred in the second phase

b = The lowest dose at which death occurred in the 2nd phase

Determination of venom LD_{50}: The LD_{50} and LD_{100} of *E. ocellatus* venom were determined by administering graded intraperitoneal doses to rats (n = 5/group). Mortality after 24 hrs was recorded, and LD_{50} was estimated using probit analysis[11].

***In vivo* antivenom evaluation of crude extract:** Twenty-four rats were divided into six groups (n = 4). The venom control received LD_{100} venom only, while treatment groups received the venom followed 30 min later by CMECNR (300 or 500 mg/kg, orally) or standard antiserum (1 mL per 0.25 mg venom, i.v.). Control groups received extract or distilled water alone[12].

***In vivo* antivenom evaluation of extract fractions:** Twenty-eight rats (n = 4/group) were injected with LD_{100} venom, followed 30 min later by administration of n-hexane, ethyl acetate, n-butanol, or aqueous fractions (300 mg/kg). Antiserum served as a positive control. Mortality and survival time were recorded for 24 hrs[12].

Hepato- and nephro-protective studies: Rats (n = 5/group) were divided into normal, venom control, ASV-treated, and n-hexane fraction-treated (300 mg/kg) groups. After 24 hrs, blood samples were collected for biochemical assays of liver and kidney function using Randox diagnostic kits.

Evaluation of haemostatic parameters: Rats (n = 5/group) were categorized as normal, venom control, ASV-treated, and n-hexane fraction-treated (300 mg/kg). Bleeding time, clotting time, and plasma fibrinogen were assessed 2-4 hrs post-treatment using standard procedures[12-14].

Antihaemorrhagic activity: The minimum haemorrhagic dose (MHD) was the lowest venom concentration causing a 10 mm lesion within 6 hrs. Lesion diameters were measured, and percentage inhibition was calculated relative to the venom control[12].

Antinecrotic activity: The minimum necrotizing dose (MND) was the venom dose producing a 5 mm lesion after 72 hrs. Lesion sizes were recorded after three days, and inhibition (%) was determined relative to control values[12].

Antihaemolytic activity: Erythrocyte suspensions (1%) were incubated with venom (0.1 mg/mL) at 37°C for 30 min, and the absorbance of the supernatant was read at 540 nm. For inhibition assays, venom was pre-incubated with n-hexane fraction (0.5 mg/mL)[15]. Percentage haemolysis and protection were calculated as follows:

$$\text{Hemolysis (\%) } Y = \frac{AT}{AC}$$

$$\text{Protection (\%)} = 100\text{-}Y$$

Where:
AT = Absorbance of treated sample
AC = Absorbance of control

Statistical analysis: All tests were conducted in triplicate. Data were expressed as Mean±SD and analyzed using one-way ANOVA followed by Tukey's *post hoc* test ($p<0.05$). Statistical analyses were performed with SPSS version 20.

RESULTS

Acute toxicity (LD_{50}): The acute oral toxicity test of the CMECNR revealed no observable toxicity or mortality in rats, even at a dose of 5000 mg/kg as presented in Table 1. Treated animals remained healthy, showing normal behavior and weight gain as shown in Fig. 1 and 2, comparable to the control group.

Lethality of *Echis ocellatus* venom: Probit analysis revealed that the LD_{50} and LD_{100} of *Echis ocellatus* venom were 0.316 mg/kg body weight and 3.55 mg/kg body weight, respectively. Mortality increases in a dose-dependent manner within 24 hrs of injection, as shown in Table 2 and Fig. 3.

***In vivo* antivenom activity of crude extract:** Rats given the LD_{100} dose of venom showed complete mortality with a mean survival time of 2.46±0.10 hrs. Treatment with CMECNR significantly ($p<0.05$) extended survival in a dose-dependent manner, with the 500 mg/kg dose offering 75% protection (MST = 20.11±2.28 hrs), similar to the standard antivenom, which achieved full survival (MST = 24.00±0.00 hrs) (Table 3).

***In vivo* antivenom activity of extract fractions:** The n-hexane fraction (n-HF) of CMECNR showed the most significant antivenom effect (MST = 22.88±2.28 hrs; 75% survival), comparable to ASV (MST = 24.00±0.00 hrs; 100% survival). Ethyl acetate and n-butanol fractions demonstrated moderate antivenom effects, while the aqueous fraction had minimal protection (Table 4).

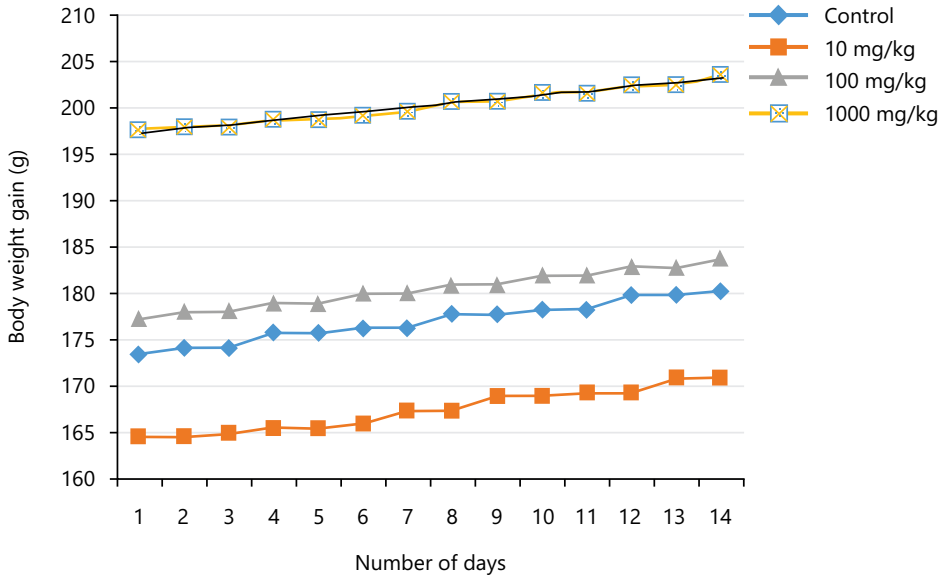

Fig. 1: Changes on body weight of rats during acute toxicity assay of CMECNR (Phase I)

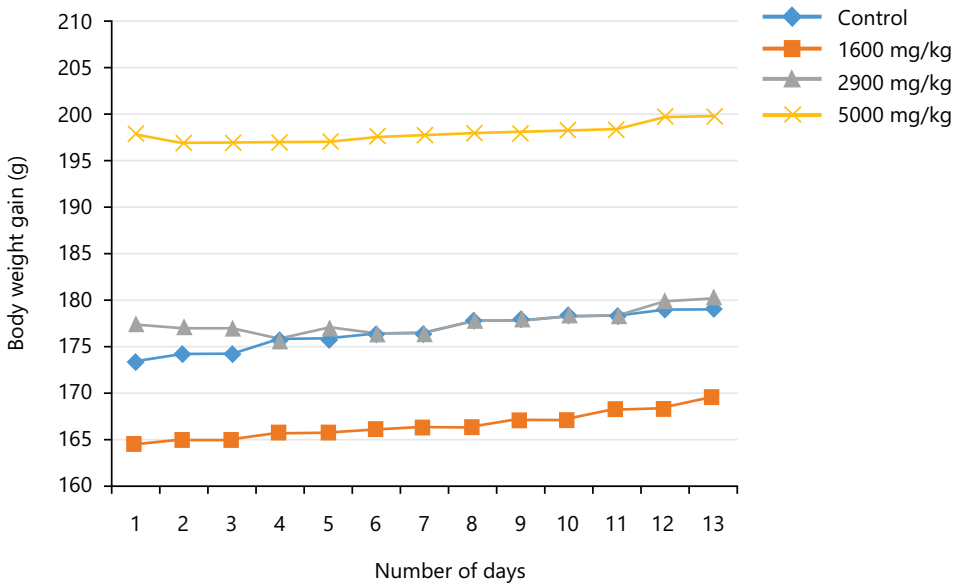

Fig. 2: Changes in body weight of rats during acute toxicity assay of CMECNR (Phase II)

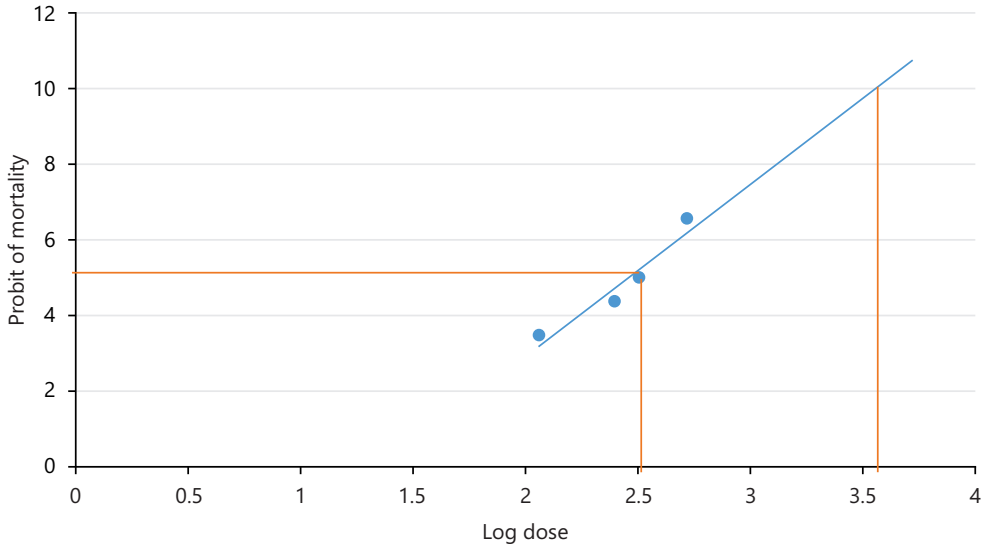

Fig. 3: Probit curve for determination of venom LD_{50} and LD_{100}

Table 1: Acute lethal effect of crude methanol extract of *Catunaregam nilotica* root-bark

Experiment	Dose	Mortality
Phase I	10	0/3
	100	0/3
	1000	0/3
Control	Normal saline	0/3
Phase II	1600	0/1
	2900	0/1
	5000	0/1
Control	Normal saline	0/1

Table 2: Probit analysis for determination of venom LD_{50} and LD_{100}

Groups	Average albino rat weight (g)	Venom dose (mg/kg b.wt)	Average venom administered (µg/kg b.wt)	Log dose	No of death/ No of rats used	Death (%)	Corrected (%)*	Probit of Mortality
1	150.4	-	-	-	0/4	0	0	-
2	115.2	1	115.2	2.06	0/4	0	*6.25	3.45
3	122.2	2	244.4	2.40	1/4	25	25	4.33
4	109.1	3	327.3	2.51	2/4	50	50	5.00
5	130.5	4	522	2.72	4/4	100	93.75	6.55

Table 3: Protective effect of CMECNR on survival in *Echis ocellatus* envenomed rats

Groups	Treatment	Extract (mg/kg b.wt)	Venom (mg/kg b.wt)	Standard antivenin (1 mL/0.25 mg venom)	Survival/ Total rats	Survival (%)	Mean survival time (hours)
1	Control (1% tween 80)	-	3.55	-	4/4	100	24.00 ± 0.00^{c}
2	Venom only	-	3.55	-	0/4	0	2.46 ± 0.10^{a}
3	Venom+extract	300	3.55	-	1/4	25	15.43 ± 2.94^{b}
4	Venom+extract	500	3.55	-	3/4	75	20.11 ± 2.28^{c}
5	Extract only	500	3.55	-	4/4	100	24.00 ± 0.00^{c}
6	Venom+ASV	-	3.55	1 mL/0.25 mg venom	4/4	100	$24.00+0.00^{c}$

Mean survival times were presented as Mean±SEM (n = 4), mean survival times carrying different superscripts are significantly ($p<0.05$) different and -: Not administered

Table 4: Anti-venom activity of CMECNR fractions on *Echis ocellatus* venom

Groups	Treatment	Extract (mg/kg b.wt)	Venom (mg/kg b.wt)	Standard antivenin (1 mL/0.25 mg venom)	Survival/ Total rats	Survival (%)	Mean survival time (hours)
1	Control (1% tween 80)	-	3.55	-	4/4	100	24.00 ± 0.00^{c}
2	Venom only	-	3.55	-	0/4	0	1.40 ± 0.18^{a}
3	Venom+n-HF	300	3.55	-	3/4	75	22.88 ± 2.28^{c}
4	Venom+EAF	300	3.55	-	2/4	50	14.61 ± 5.42^{b}
5	Venom+n-BF	300	3.55	-	1/4	25	11.39 ± 4.41^{b}
6	Venom+AF	300	3.55	-	0/4	0	6.72 ± 0.57^{ab}
7	Venom+ASV	-	3.55	1 mL/0.25 mg venom	4/4	100	24.00 ± 0.00^{c}

n-HF: n-Hexane fraction, EAF: Ethyl acetate fraction, n-BF: n-Butanol fraction, AF: Aqueous fraction, ASV: Anti-snake venom, Mean survival times were presented as Mean±SEM (n = 4), mean survival times carrying different superscripts are significantly ($p<0.05$) different and -: Not administered

Table 5: Effect of *Catunaregam nilotica* root-bark n-hexane fraction on liver function indices of *Echis ocellatus* envenomed albino rats

	Normal control	Venom control	Venom+ASV	Venom+n-HF
Total protein (g/L)	13.34 ± 0.44^{d}	7.10 ± 0.25^{a}	8.65 ± 0.25^{b}	10.06 ± 0.36^{c}
Albumin (g/L)	07.75 ± 0.04^{d}	05.24 ± 0.10^{a}	06.69 ± 0.07^{c}	05.86 ± 0.11^{b}
Total Bilirubin (mg/dL)	00.87 ± 0.05^{b}	01.93 ± 0.57^{c}	00.69 ± 0.08^{a}	00.79 ± 0.06^{ab}
Direct Bilirubin (mg/dL)	00.32 ± 0.03^{a}	00.72 ± 0.02^{d}	00.37 ± 0.02^{b}	00.48 ± 0.01^{c}
AST activity (U/L)	13.55 ± 0.07^{a}	46.93 ± 0.72^{c}	18.71 ± 0.11^{b}	19.53 ± 0.43^{b}
ALT activity (U/L)	29.76 ± 0.44^{b}	69.87 ± 0.52^{c}	24.93 ± 0.63^{a}	30.27 ± 0.50^{b}
ALP activity (U/L)	170.67 ± 0.88^{b}	240.00 ± 1.73^{d}	166.67 ± 0.58^{a}	181.60 ± 1.73^{c}

Results are presented as Mean±SEM (n = 4), values carrying different superscripts from the normal control across the row are significantly ($p<0.05$) different using ANOVA and Duncan multiple range test

Table 6: Effect of *Catunaregam nilotica* root-bark n-hexane fraction on renal function indices of *Echis ocellatus* envenomed albino rats

	Normal control	Venom control	Venom+ASV	Venom+n-HF
Urea (mmol/L)	05.90 ± 0.14^{a}	07.96 ± 0.14^{c}	06.19 ± 0.09^{a}	07.52 ± 0.09^{b}
Cretinine (µmol/L)	99.01 ± 0.82^{a}	125.00 ± 1.63^{d}	114.25 ± 2.06^{c}	105.50 ± 1.73^{b}
Uric acid (mmol/L)	00.49 ± 0.02^{a}	00.51 ± 0.01^{a}	00.59 ± 0.02^{a}	00.47 ± 0.03^{a}
Na (mmol/L)	147.28 ± 0.03^{d}	146.43 ± 0.06^{c}	145.57 ± 0.06^{b}	142.27 ± 0.03^{a}
K (mmol/L)	03.63 ± 0.05^{a}	4.51 ± 0.03^{d}	04.30 ± 0.02^{c}	04.20 ± 0.02^{b}
Cl^{-} (mmol/L)	104.33 ± 33^{a}	104.50 ± 0.07^{b}	104.54 ± 0.01^{b}	104.71 ± 0.03^{c}

Results are presented as Mean±SEM (n = 4), values carrying different superscripts from the normal control across the row are significantly ($p<0.05$) different using ANOVA and Duncan multiple range test

Effect on hepatic function: Envenomation caused a significant ($p<0.05$) elevation in hepatic enzymes (AST, ALT, ALP) and bilirubin levels, alongside a decline in total protein and albumin. Treatment with n-HF effectively restored these indices toward normal values, comparable to ASV (Table 5).

Effect on renal function: Venom administration led to elevated urea, creatinine, and potassium concentrations. Treatment with n-HF significantly ($p<0.05$) decreased creatinine and potassium levels more effectively than ASV, while both treatments normalized urea and sodium concentrations. Uric acid and chloride levels remained unchanged (Table 6).

Table 7: Effects of *Catunaregam nilotica* root-bark n-hexane fraction on hemostatic parameters in *Echis ocellatus*-envenomed rats

Treatment group	Bleeding time (s)	Clotting time (s)	Defibrinogenating effect
Normal control	63.75±0.75^{a}	93.75±1.55^{a}	63.75±1.50^{a}
Venom control	128.00±1.08^{d}	195.50±1.66^{c}	NC
Venom+ASV	79.50±1.70^{b}	115.00±1.78^{b}	120.25±1.38^{b}
Venom+n-HF	90.75±2.39^{c}	119.50±0.87^{b}	124.00±1.67^{b}

Results are presented as Mean±SEM (n = 4), values with different superscripts across rows differ significantly ($p<0.05$) and NC: No clotting

Table 8: Effects of *Catunaregam nilotica* root-bark n-hexane fraction on hemorrhagic and necrotic lesions in *Echis ocellatus*-envenomed rats

Treatment group	Hemorrhagic lesion (mm)	Necrotic lesion (mm)
Normal control	0.00±0.00^{a}	0.00±0.00^{a}
Venom control	11.05±0.01^{d}	16.63±0.94^{c}
Venom+ASV	2.33±0.08^{b}	5.78±0.88^{b}
Venom+n-HF	4.23±0.20^{c}	7.22±0.60^{b}

Results are presented as Mean±SEM (n = 4), Different superscripts indicate significant differences ($p<0.05$)

Table 9: Effects of *Catunaregam nilotica* root-bark n-hexane fraction on hemolysis in *Echis ocellatus*-envenomed rats

Treatment group	Hemolysis (%)
Normal control	2.60±0.16^{a}
Venom control	90.70±2.18^{d}
Venom+ASV	25.18±0.71^{b}
Venom+n-HF	29.69±1.61^{c}

Results are presented as Mean±SEM (n = 4), values with different superscripts differ significantly ($p<0.05$)

Effects on hemostatic parameters: *Echis ocellatus* envenomation significantly prolonged bleeding (128.00±1.08 sec) and clotting (195.50±1.66 sec) times, indicating coagulopathy. Administration of n-HF markedly shortened these times (90.75±2.39 and 119.50±0.87 sec, respectively), comparable to ASV-treated rats. The defibrinogenating effect, absent in venom-only rats, was restored by both treatments (Table 7).

Antihaemorrhagic and antinecrotic effects: Venom injection produced severe hemorrhagic (11.05±0.01 mm) and necrotic (16.63±0.94 mm) lesions. Treatment with n-HF significantly ($p<0.05$) reduced lesion diameters (4.23±0.20 and 7.22±0.60 mm, respectively), comparable to ASV (2.33±0.08 mm and 5.78±0.88 mm) (Table 8).

Antihaemolytic activity: Venom exposure caused extensive hemolysis (90.70±2.18%). Treatment with n-HF markedly reduced hemolysis to 29.69±1.61%, comparable to ASV (25.18±0.71%). This indicates strong erythroprotective and membrane-stabilizing potential of the n-HF fraction (Table 9).

DISCUSSION

The crude methanol extract of *Catunaregam nilotica* root bark showed no signs of toxicity or mortality up to 5000 mg/kg, indicating a broad safety margin and classifying it as practically non-toxic according to OECD (2001) standards. This observation agrees with findings from other Rubiaceae species[16]. The LD_{50} and LD_{100} values of *Echis ocellatus* venom were consistent with previous reports[17,18]. Minor differences in venom potency reported may reflect geographical and ecological variations in venom composition[19]. *In vivo* studies revealed that *E. ocellatus* envenomation caused typical viperid symptoms, hemorrhage, necrosis, coagulopathy, and organ dysfunction. Treatment with the crude extract of *C. nilotica* improved survival and reduced tissue injury, suggesting the presence of antivenom compounds. Among the solvent fractions, the n-hexane fraction (n-HF) was the most effective, similar to reports on *Azadirachta indica* and *Albizia chevalieri*[9,19]. Its strong activity may be linked to non-polar terpenoids, steroids, and alkaloids that chelate metal ions, inhibit venom enzymes, and reduce oxidative stress[20]. Envenomed rats exhibited liver and kidney damage, evidenced by increased transaminases, urea, creatinine, and electrolyte imbalance consistent with SVMP- and PLA_2-mediated cytotoxicity[21]. Treatment with n-HF normalized these

iochemical parameters, indicating strong hepatoprotective and nephroprotective effects rarely observed with standard antivenoms[22,23]. Similar recovery has been reported with plant-based antivenoms such as *Moringa oleifera*, *Annona senegalensis*, and *Andrographis paniculata*[24-27]. The presence of flavonoids, tannins, and terpenoids in n-HF supports its antioxidant, anti-inflammatory, and protease-inhibitory mechanisms[3,6]. Venom exposure also caused hemostatic disturbances, including prolonged clotting time, defibrinogenation, and local hemorrhage. The n-HF treatment restored normal coagulation, reduced lesion size, and prevented fibrinogen depletion, likely through inhibition of zinc-dependent SVMPs responsible for vascular and extracellular matrix damage[28,29]. Its suppression of hemolysis further suggests Phospholipase A_2 inhibition, preventing erythrocyte lysis and lipid peroxidation[30-33].

CONCLUSION

The n-hexane fraction of *Catunaregam nilotica* root bark demonstrated potent antivenom activity against *Echis ocellatus* venom by effectively attenuating both systemic and local toxic effects. Treatment significantly improved survival, normalized hepatic and renal biomarkers, restored hemostatic balance, and reduced venom-induced hemorrhagic and necrotic lesions. These findings substantiate the therapeutic relevance of *C. nilotica* and highlight its potential as a complementary phytotherapeutic agent in snakebite management.

SIGNIFICANCE STATEMENT

Snakebite envenomation by *Echis ocellatus* remains a major cause of morbidity and mortality in Sub-Saharan Africa. The present findings confirm that the n-hexane fraction of *Catunaregam nilotica* root bark effectively counteracts venom-induced hemorrhage, necrosis, hemolysis, and organ damage. Unlike conventional antivenoms that primarily neutralize systemic toxins, this plant-derived fraction offers protection against both local and systemic pathologies. The study provides scientific validation for its traditional use and supports its potential development as an affordable, complementary antivenom therapy.

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