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Neutralization and Hematological Recovery Following *Echis Ocellatus* Envenomation: Comparative Efficacy of Species-Specific Antibody and Standard Antisnake Venom

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ABSTRACT

Background: Envenomation by *Echis ocellatus* remains a significant health concern due to its potent venom and re- classified as a neglected tropical disease by the World Health Organization (WHO), pose significant health risks, particularly in rural Sub-Saharan Africa. This study evaluates the immune response of antibody raised against *Echis ocellatus* venom, their protein characterization, and their efficacy in neutralizing its venom toxicity.

Results: Protein quantification revealed varying concentrations, indicating variability in immune response. SDS-PAGE confirmed the presence of intact IgG antibodies (~150 kb) and venom proteins, including metalloproteinases, phospholipases, and serine proteinases. Lethality studies established an LD_{50} of 0.77 mg/kg, with regional variations noted in venom potency and dose-dependent mortality increasing up to 100% at 1.2 mg/kg. The *Echis ocellatus* antibody effectively neutralized the venom at $2LD_{50}$, with an ED_{50} of 0.73 mg/kg, demonstrating superior efficacy over standard Antisnake Venom (ASV) in mitigating hematoxic effects. Neutralization experiments showed 100% survival at 20–50 mg/kg antibody doses.

Hematological analysis revealed venom-induced reductions in WBC, RBC, hemoglobin, hematocrit, and platelet levels, indicating immunosuppression, hemolysis, and thrombocytopenia. Treatment with the Echis ocellatus antibody showed enhanced recovery of RBC, hemoglobin, and platelet levels, while standard ASV was more effective in restoring WBC counts.

Hematological analysis demonstrated venom-induced hematotoxicity, with significant reductions in WBC, RBC, hemoglobin, hematocrit, and platelet levels, indicating immunosuppression, hemolysis, and thrombocytopenia. However, *Echis ocellatus* antibody showed enhanced recovery of these parameters, outperforming the standard antivenom in restoring hematological balance. Time-dependent analysis showed enhanced recovery at 36 hours compared to 24 hours, suggesting progressive neutralization.

Conclusion: The *Echis ocellatus* antibody demonstrated dose-dependent venom neutralization, enhancing survival and reducing hematological damage. These results support its potential as an alternative or complement to standard antisnake venom.

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Keywords: *Echis ocellatus*, WHO, venom toxicity, standard ASV, antibody neutralization, hematological parameters, snakebite envenomation.

INTRODUCTION

Snakebite envenoming represents a major health problem in tropical and subtropical countries (Bailon Calderon *et al.*, 2020). Considering the elevated number of accidents and high morbidity and mortality rates, the World Health Organization reclassified this disease to category A of neglected diseases (Senthilkumaran *et al*., 2023). Each year, approximately 5.4 million snake bites occur globally, resulting in 1.8 to 2.7 million cases of envenomation (Du *et al*., 2024**).** This public health crisis leads to more than 100,000 deaths and over 400,000 cases of morbidity**,** underscoring the urgent need for increased awareness and intervention (Alsolaiss *et al*., 2024).

Among the medically significant snakes, *Echis ocellatus*, also known as the carpet or saw-scaled viper, poses a considerable public health threat, particularly in West Africa and Nigeria (Tijani *et al*., 2024). This species is responsible for the highest casualty rates, largely due to its highly toxic venom, which is notorious for causing severe bleeding and hypocoagulability (Larréché *et al*., 2024**).** The venom's pathology is characterized by critical coagulation and inflammatory disturbances 16,17, primarily attributed to several major toxin components: Metalloproteinases (34.84%)**,** Phospholipase A2s (21.19%)**,** and Serine proteases (15.50%) (Dingwoke *et al*., 2021); Subsequently, disrupting hemostasis and inflicting tissue damage (Larréché *et al*., 2021), initiating both local and systemic inflammatory responses (Cavalcante *et al*., 2022).

Clinically, snake bite envenomation is characterized by pathophysiology manifestation with victims experiencing local effects including inflammation**,** edema**,** hemorrhage**,** and myonecrosis**:** In contrast, systemic effects can include hemorrhage**,** consumptive coagulopathy**,** shock, and acute kidney failure (Fernandes *et al*., 2024**).** The rapid onset of local symptoms can be accompanied by alarming systemic signs, which may lead to serious complications such as organ damage, tissue loss, and even the necessity for amputations. Without prompt and effective medical intervention, snake bites can result in fatal outcomes (Nandana *et al*., 2024).

The key protein families in the venom of *E. ocellatus* play crucial roles in this envenomation process (Offor and Piater, 2024). Snake Venom Metalloproteinases (SVMPs) compromise the integrity of blood vessel membranes and disrupt normal coagulation pathways (Dingwoke *et al*., 2024). Snake Venom Serine Proteases (SVSPs) are involved in the depletion of fibrinogen, significantly impairing hemostatic function (Silva *et al*., 2021). In addition, Phospholipase A2 (PLA2) enzymes contribute to hemolysis and anticoagulation, further complicating the clinical scenario (Xie *et al*., 2020).

While antivenom therapy is a critical treatment option for mitigating systemic symptoms of envenomation, it often faces limitations when addressing local effects (Huertas *et al*., 2023; Silva *et al*., 2023). The antibody effectively bound to various toxin variants, preventing their interaction with the receptor and offering protection against lethal venom exposure. Structural analysis revealed that its binding mechanism replicates the receptor-toxin interaction, supporting the development of antibody-based universal antivenoms for snakebite treatment (Khalek *et al*., 2024; Biruš *et al*., 2025). However, conserved antigenicity of key toxic components across venoms supports the potential development of a highly potent, regionally effective viper-specific antivenom with broader therapeutic coverage (Lim *et al*., 2024; Khochare *et al*., 2024). The venom composition can differ significantly between snake populations due to geographical, genetic, ecological, and environmental factors, resulting in variations in toxin profiles which will equally affect the efficacy of antivenom products (Ruiz-Campos *et al*., 2021; Alomran *et al*., 2022). This can lead to cases where antivenoms produced using venom from one region show reduced efficacy against venom from a different geographical area (Youngman *et al*., 2022; Tan *et al*., 2022). Additionally, commercially available polyvalent antisnake venoms (ASV) are designed to neutralize venoms from multiple snake species, which can introduce cross-reactivity limitations (Chaisakul *et al*., 2020; Bailon Calderon *et al*., 2020). These ASV may not contain sufficient antibody titers against *Echis ocellatus* venom-specific toxins, leading to suboptimal neutralization (Lim *et al*., 2023). Moreover, the administration of ASV is associated with potential adverse reactions, including serum sickness, anaphylaxis, and hypersensitivity responses, which further complicate treatment

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(Rey-Suárez and Lomonte, 2020). As a result, treatment outcomes may vary widely, with some patients experiencing incomplete neutralization, prolonged hospital stays, or persistent coagulopathy despite receiving standard antivenom therapy (Isbister, 2024).

Species-specific antibodies represent a promising therapeutic advancement in snakebite treatment (Lee *et al*., 2020). Unlike polyvalent ASV, which contain antibodies against multiple snake species (Alomran *et al*., 2021; Tan *et al*., 2021). Subsequently, monospecific antibodies are developed exclusively to neutralize toxins from a single species by recognizing and binding to key toxic components of the venom with high specificity (Karim-Silva *et al*., 2020). Due to intraspecific venom variation with venom lethality varied with geographical origins of the snake, neutralization efficacy varied vastly with normalized potency values presumably due to the compositional differences of dominant proteins in the different venoms of same species; hence, antivenom use should be optimized in different geographical areas (Hia *et al*., 2020). This targeted approach minimizes the risk of insufficient neutralization and reduces the likelihood of delayed or prolonged envenomation symptoms resulting to its improved stability and reduced degradation, ensuring higher potency and longer-lasting therapeutic effects enhancing patient safety (Machado Marinho *et al*., 2024).

The specific aims and objectives of this research are to:

- ✓ Toxicity assessment: Determination of the median lethal dose (LD_{50}) of *Echis ocellatus* venom.
- ✓ Antibody characterization: Evaluation of the integrity and stability of the species-specific antibody using SDS-PAGE analysis.
- ✓ Neutralization efficacy: Determination of the effective dose (ED_{50}) and analysis of neutralization at $2LD_{50}$.
- ✓ Hematological impact: Examination of venom-induced alterations in blood parameters, including white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), and platelet (PLT) levels.

Despite the known implications of these hematological parameters, there remains a gap in understanding their temporal dynamics following *Echis ocellatus* envenomation. This study aims to elucidate the changes in key hematological parameters post-envenomation. Assessing the significance of these changes can enhance the understanding of venom-induced hematological alterations and their potential implications for patient management and treatment strategies. The findings of this study will contribute to the broader knowledge of snakebite pathology and may inform clinical practices to improve outcomes for affected individuals.

MATERIALS AND METHODS

Venom Source And Choice

The lyophilized venom was acquired from the venom bank at Bayero University Kano, located in Kano State. The venom was sourced from *Echis ocellatus* snake in the northeastern region of Nigeria.

Experimental Animals

Five male rabbits weighing between 2.0Kg (2000g) and 2.4Kg (2400g); and one hundred and ten male and female Swiss mice weighing between 16g and 27g where purchased and were bred at the Vivarium of the Isogenic Mice Centre within the Department of Pharmacology, Aminu Kano Teaching Hospital, Bayero University Kano (BUK) Nigeria. The animals were housed in controlled rooms and provided with clean tap water and commercial feeds (*Vital Feed*, Plateau State, Nigeria) ad libitum until they were used. They were allowed to adapt to the environment for two weeks before the commencement of the experiment. All animals were handled according to the guidelines for research and evaluation of traditional medicine using animal model (WHO, 2000) and the international guiding principles for biomedical research involving animals

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(CIOMS, 1985). Ethical clearance was granted by the Headship of the Ministry of Defence Health Regulation Ethical Committee Protocol (MODHREC) NHREC/MOD-HREC/15/02/23/C… MOD/SC/HREC/1/121.

Treatment of the Rabbits For Immunization

Immunization protocol

Immunogen Preparation

The immunogens used for rabbit immunization were prepared under aseptic conditions, as previously described by Gómez *et al*. (2022). The *Echis ocellatus* venom was mixed with Freund's complete adjuvant (or incomplete Freund's adjuvant) (sigma aldrich) in a 1:1 (v/v) ratio. The immunogen was kept on ice until it was used as described below

Echis ocellatus Venom Dilution Preparation

0.002g of *Echis ocellatus* venom was weighed with an electronic weigh balance (Kern and Sohn EMB 6002-2, Germany) added to 1ml of distilled water (D/W) to make 2000µg/ml of stock venom. 400µg/ml of *Echis ocellatus* venom solution was made by added 4ml of D/W. 150µg/ml of the *Echis ocellatus* venom was prepared by using 0.375 mL of the 400 µg/mL *Echis ocellatus* venom solution was used throughout the immunization. 1:1 ratio of 0.375ml of 400 µg/mL of *Echis ocellatus* venom solution was mixed with 0.375 mL of complete Freund's adjuvant (CFA) for the initial immunization. For subsequent booster injections, mix the 0.375ml of 400 µg/mL of *Echis ocellatus* venom solution with incomplete Freund's adjuvant (IFA).

Immunization

Immunization was carried out using the low dose, low volume immunization protocol described by Darkaoui *et al*. (2024). The first week was Acclimatization of rabbits. Second week was the 1st immunization with 0.375ml of 400 µg/mL of *Echis ocellatus* venom solution was mixed with 0.375 mL of complete Freund's adjuvant (CFA). Subsequently, with two weeks intervals, the 2nd and 3rd immunization was done with 0.375ml of 400 µg/mL of *Echis ocellatus* venom solution with incomplete Freund's adjuvant (IFA). The last immunization was given at the 8th week from the start of immunization with a booster dose of 0.375ml of 400 µg/mL of *Echis ocellatus* venom solution. After a week interval, blood samples were collected at the end of the immunization process as adopted and modified by Ratanabanangkoon *et al*. (2016). Sera were obtained by centrifuging the clotted blood at 800 x g for 15 minutes at room temperature. The sera were then stored at -20°C until used.

Echis ocellatus Antibody Partial Purification

Echis ocellatus antibody was purified using ammonium sulfate (Sigma Aldrich) precipitation and gel filtration chromatography. One gram (1g) of Sephadex C50 (Lifescience UK) was dissolved in 20ml of PBS (Phosphate Buffered Saline) (VWR amresco lifescience) and allowed to stand for 24 hours. The solution was then packed into a burette with PBS solution covering the surface of the column to allow for elution. The *Echis ocellatus* antibody serum mixture was gradually poured into the column, and the eluent was collected. The eluent was subjected to 12% sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE). Protein bands was stained with Coomassie brilliant blue and subjected to computerized densitometric analysis. The protein concentration was determined using the biuret method (Kpordze *et al*., 2024).

Protein Concentration Determination (Biuret Method)

Twenty microliters (20 µl) of the *Echis ocellatus* antibody mixture was added to one hundred eighty microliters (180 µl) of biuret reagent (Bio basic Inc. Canada). The mixture was incubated for 5 minutes at 37°C. The absorbance was measured at a wavelength of 550 nm using a 96-well plate and a microplate reader. The protein concentration was extrapolated from the standard protein curve using the equation: $y = 0.0081x – 0.0125$ (Zhang *et al*., 2023).

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Lethality Studies

Half Lethality Dose (LD_{50})

The stock solution of *Echis ocellatus* venom 1mg/ml was prepared by weighing 0.01g and dissolving in 1ml PBS solution. The rabbits were randomly separated into 8 groups of 3 albino mice per group. Each mouse in 8 different were treated with (0.1, 0.3, 0.5, 0.7, 0.9, 1.3 and 1.5) mg/ml per body weight of *Echis ocellatus* venom. According to their weigh, each group were injected Intraperitoneal (IP) different doses of *Echis ocellatus* venom: as shown in the formula below, the volume of the dose administered was calculated following the steps

Volume (mL)=Dose (mg/kg)×Animal weight (kg)Concentration (mg/mL)

The mice were observed continuously for the first 2 hours post-injection, then at intervals for up to 24–72 hours. The number of deaths were recorded in each dose group modified from Dearden and Hewitt, (2021).

For lethality studies (Neutralization)

The dose findings were determined by incubating both $2LD_{50}$ (twice the lethal dose) *Echis ocellatus* venom and the volume of *Echis ocellatus* antibody for 30 minutes before injecting them into the mice.

The mice are weighed, grouped from (1-3) with concentration of 20, 30 and 40 µL/g respectively. The volume of the antibody to be administered were calculated using the formula with antibody concentration 10.06mg/ml.

$$\text{Volume to be administered (ml)} = \frac{Weigh\ of\ mice\ cdose\ required}{echis\ ocellatus\ \text{antibody concentration}}$$

The volume of the $2LD_{50}$ *Echis ocellatus* venom to be administered will be calculated using the formula

Volume to be administered for $2LD_{50}$ = $2LD_{50}$ × weigh of mice

The dose each for *Echis ocellatus* venom and antibody were incubated together for 30 minutes at 37ºC in the incubator. The mice were injected Intraperitoneal (IP) respectively according to their weigh. The mice were then observed for 24 hours for mortality.

Neutralization assay

The albino mice were randomly separated into 6 groups of 3 per group for the treatment with the *Echis ocellatus* antibody. The groups were labeled group 1-6. Each mouse in Groups 1 -6 were injected Intraperitoneal (IP) with incubated $2LD_{50}$ *Echis ocellatus* venom and (5, 10, 20, 30, 40 and 50 µL/g) concentration *Echis ocellatus* antibody dose. They were observed for 36 hours for mortality according to Li *et al*. (2024).

Hematological assay

The mice were grouped into groups. Group 1 were given normal saline (N/S), group 2 were give $2LD_{50}$ *Echis ocellatus* venom dose; Group 3 were given $2LD_{50}$ dose + *Echis ocellatus* antibody and $2LD_{50}$ dose + standard antisnake venom (Bharat Serums and Vaccines india) respectively in doses of 30, 40, and 50 µL/g respectively. The groups were monitored for 24 hours. Whole blood samples from the mice used to perform the Full Blood Count (FBC) parameters using the automated hematolyzer (Mindray automated analyzer Japanase).

Estimation Of Full Blood Count (FBC)

FBC was determined by flow cytometry method (Akorsu *et al*., 2023).

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FBC was determined accordingly to protocol for Heamtology analyzer using Mindray. The knob was turn on from the back of the analyzer and allowed to initiate for 10 minutes and daily maintainance was performed. The controls were brought forward and allowed to attain room temperature. The controls were analyzed by allowing the probe to pick 50µL from the control bottle and analyzed. The result was displayed on the screen of the system and recorded

Data Analysis

The data obtained from the study were carefully subjected to statistical analysis using ANOVA with Tukey multiple comparison post-test by computer statistical software (GraphPad InStat Version 3.10, 32 bit for Windows, by GraphPad Software Inc., USA). Results were expressed as mean and standard deviation (Mean ± SD). Level of significance of difference between means was considered at $P < 0.05$.

RESULTS

Result of Partial Purification *Echis Ocellatus* Antibody.

SDS-PAGE

The SDS-PAGE analysis revealed a prominent band at approximately 150kb in antibody samples A1, A2, and A4, corresponding to intact IgG antibodies, with additional faint bands at ~50kb and ~25kb**,** likely due to partial fragmentation into heavy and light chains. The intensity of the 150kb band varied, with A2 (20:20 ratio) showing the strongest signal, reflecting higher antibody concentration. In contrast, venom samples V1 and V2 exhibited multiple distinct bands at **~**85kb, ~45kb, and ~25kb**,** representing venom proteins such as metalloproteinases, serine proteinases, and phospholipases, with no visible band at 150kb, confirming the absence of antibody.

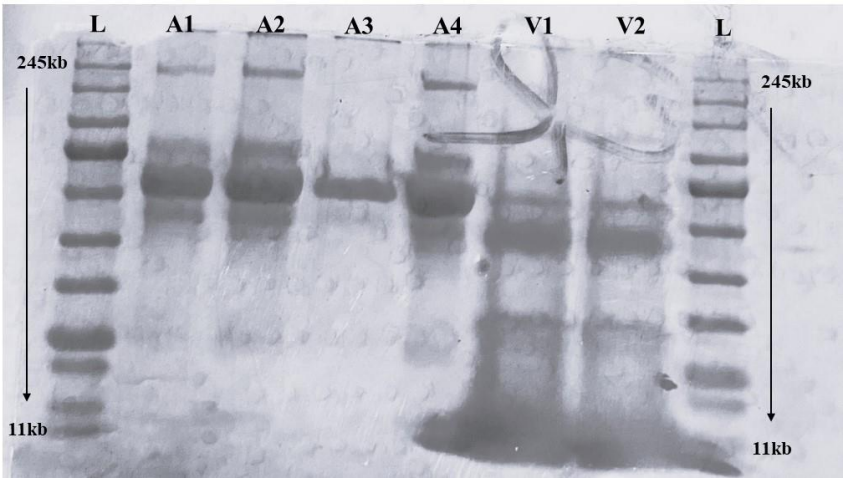

Figure 1: SDS-PAGE analysis of partially purified snake venom antibody and pure snake venom. L-protein ladder (245-11kb), Venom antibody (mixed with loading buffer in varying ratios): A1- (10:20, antibody:buffer), A2- (20:20, antibody:buffer), A3- (30:10, antibody:buffer), A4- (10:30, antibody:buffer), and V1/V2- pure venom.

Protein Concentration

The result shows absorbance values and corresponding protein concentrations obtained from immunized rabbits with *Echis ocellatus* venom. Whole Serum A, with an absorbance value of 0.069 and a protein concentration of 10.06 mg/mL, indicates a robust immune response and successful isolation of venom immunoglobulins. In contrast, Whole Serum B, with an absorbance value of 0.021 and a protein concentration of 4.20 mg/mL, suggests a weaker immune response or variations in venom dose response, possibly due to less efficient purification or inherent physiological differences between the rabbits.

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Table 1: Protein concentration *Echis ocellatus* antibody

S/No.	Whole serum	Absorbance	Protein conc. (mg/mL)
1	Whole serum A	0.069	10.06
2	Whole serum B	0.021	4.20

Lethality studies

Toxicity of *Echis ocellatus* venom

Table 2: LD_{50} response

No. of mice	3	3	3	3	3	3	3	3
Echis ocellatus venom dose (mg/mL)	0.1	0.3	0.5	0.7	0.9	1.1	1.3	1.5
Mortality	0	0	1	2	3	3	3	3
% mortality	0	0	33.3	66.7	100	100	100	100

The result of treatment with the mice with *Echis ocellatus* venom was represented in Table 2. It indicated 0% mortality in concentrations between 0.1-0.3 mg/mL *Echis ocellatus* venom dose per body weight of the mice while 0.5-0.7 mg/mL dose was 33.3-66.7% mortality rate and 0.9-1.5 mg/mL dose was 100% mortality rate.

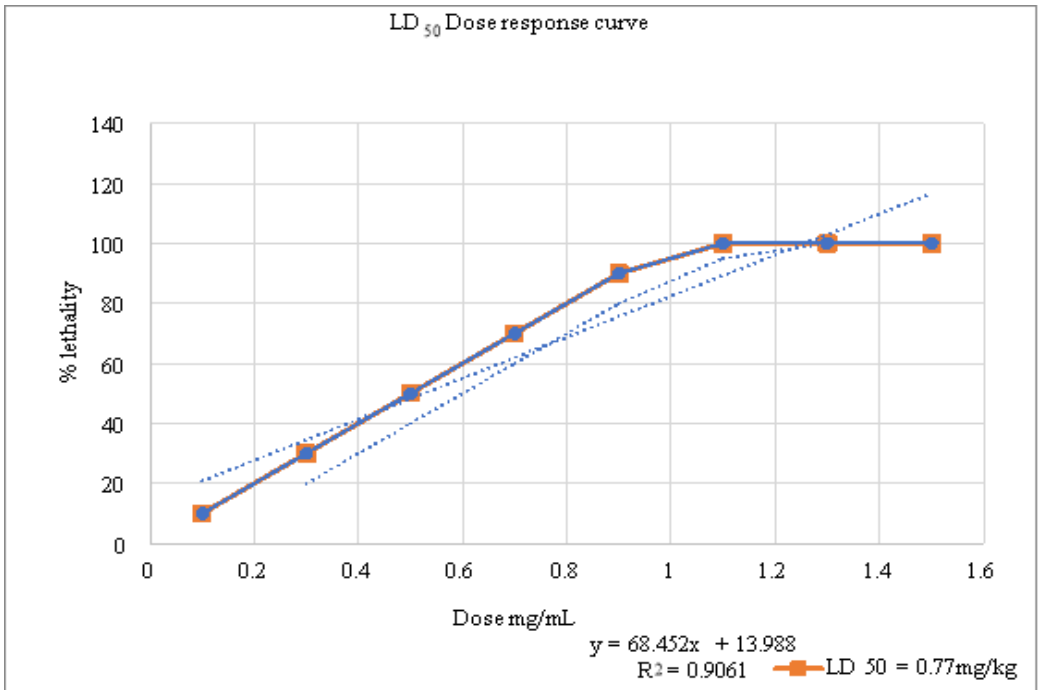

Figure 2: Dose concentration curve of *Echis ocellatus* venom

Dose-response curve was used to illustrate the relationship between the administered dose of *Echis ocellatus* venom (mg/kg) and the percentage (%) mortality observed in Figure 2. The graph demonstrates a clear dose-dependent increase in lethality, with a linear regression line (y=68.452x+13.988) indicating a strong correlation (R^2=0.9061) between dose and effect. The lethal dose for 50% of the population (LD_{50}) was determined to be 0.77 mg/kg, highlighting the venom's high toxicity. Beyond a dose of 1.2 mg/kg, lethality plateaus at 100%, suggesting saturation.

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Table 3: Dose response neutralization result

$2LD_{50}$ *Echis ocellatus* venom + *Echis ocellatus* Antibody (Dose µg/g)	5	10	15	20	25	30	35	40	45	50
No. of death out of three	3	2	1	0	0	0	0	0	0	0
Mortality %	100	60	20	0	0	0	0	0	0	0

These result shows the neutralization effect of *Echis ocellatus* antibody on double the lethal dose ($2LD_{50}$) of its venom illustrated in Table 3 with % mortality. It indicated 100% mortality in 5mg per body weight of the mice. In doses of 10-15 mg/kg there was partial mortality rate and doses of 20-50 mg/kg showed 100% protection of the *Echis ocellatus* antibody.

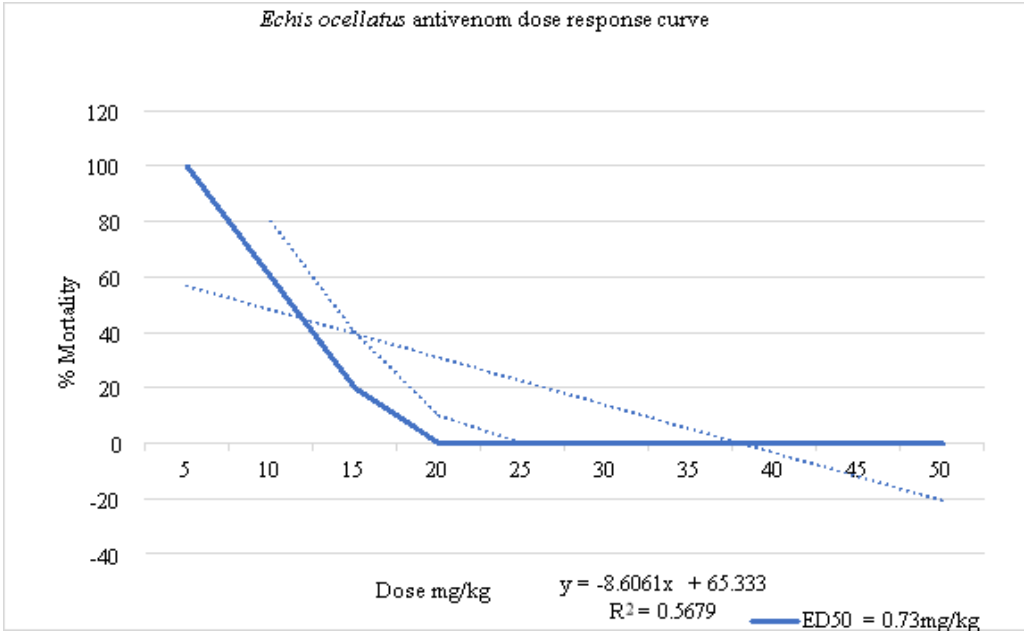

Figure 3: Dose concentration curve of *Echis ocellatus* antivenom

These shows the dose-response relationship between the administered dose of *Echis ocellatus* antivenom and the observed response Figure 3. The linear regression equation y = -8.6061x + 65.333 indicates that as the dose of antivenom increases, the mortality or toxic effect decreases, demonstrating a dose-dependent neutralization. The coefficient of determination (R^2=0.5679) suggests a moderate correlation, indicating that 56.79% of the variability in response is explained by the antivenom dose. Experimental results showed that lower doses of antivenom provide only partial neutralization, potentially leading to some toxicity. In contrast, higher doses (≥50 mg/kg) effectively neutralize the venom, achieving 100% survival within a 24-36-hour observation window.

Table 4: Effect of hematological parameters on neutralization of *Echis ocellatus* venom

			Echis ocellatus antibody			Standard antisnake venom		
	Control	2 LD_{50}	grp 1	grp2	grp 3	grp1	grp 2	grp 3
WBC	4.4	4.96	4.98	4.78	4.58	5	4.8	4.38
RBC	7.18	6.59	6.0	6.08	5.78	5.76	6.08	6.04
HGB	14.95	14.53	14.02	12.9	12.2	12.46	10.74	13.62

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HCT	37	39.79	42.2	38.6	37.6	38.4	38.6	41.0
PLT	409	302.9	190.6	173.8	232.0	171.2	196.0	185.8

These illustrates the comparative neuralization effect of hematological parameters on *Echis ocellatus* venom with both *Echis ocellatus* antibody and standard antisnake venom in Table 4. The control which is the mice treated with normal saline were within normal range of its respective parameter. The group treated with double lethal dose (2 LD_{50}) of *Echis ocellatus* venom showed visible increment in their respective parameter illustrating effect of venom toxins during envenomation. While the treatment group was gradually improving its effectiveness against neutralization effect compared to the envenomed group according to dose increment.

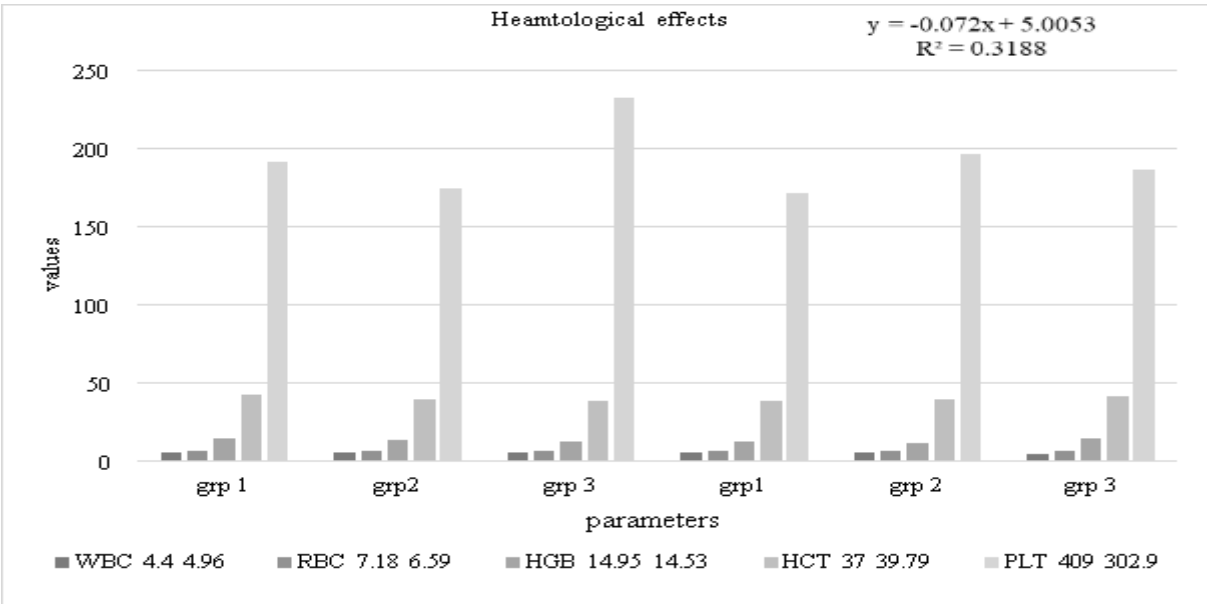

Figure 4: Hematological changes

The linear regression equation y = -0.072x + 5.0053 indicates that as the dose of antivenom increases, the toxic effect decreases, demonstrating a dose-dependent neutralization. The coefficient of determination (R^2 = 0.3188) suggests a weak correlation illustrated in Figure 4. The *Echis ocellatus* antibody shows greater efficacy in improving hemoglobin (HGB), hematocrit (HCT), and platelet (PLT) levels, particularly at higher doses, suggesting its potential in mitigating anemia and thrombocytopenia. The standard ASV achieves slightly better recovery in white blood cell (WBC) counts, especially at lower doses, indicating a stronger immunoprotective effect. Both treatments demonstrate significant mitigation of venom-induced damage, with optimal efficacy depending on the specific parameter and dose. The p-values for all parameters (WBC, RBC, HGB, HCT, PLT) are highly significant ($p=3.17\times10^{-11}$), indicating statistically significant differences between the groups (*Echis ocellatus* antibody, standard ASV, and LD_{50} venom). The *Echis ocellatus* antibody treatment demonstrated slightly higher efficacy in some hematological parameters (HGB, HCT, and PLT), suggesting its potential for mitigating venom-induced damage, while the standard ASV showed competitive recovery but was slightly less effective in certain parameters compared to the antibody.

Table 5 : FBC Parameters with time

Parameter	24 Hours Mean ± SD	36 Hours Mean ± SD	p-value
WBC	4.77 ± 0.40	4.43 ± 0.35	0.0010
RBC	6.06 ± 0.05	6.07 ± 0.10	0.0015
HGB	13.24 ± 0.68	12.47 ± 1.30	0.2378

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HCT	39.93 ± 1.97	38.00 ± 3.61	0.0300
PLT	198.80 ± 29.95	189.00 ± 22.03	0.6553

In Figure 4.7, the mean WBC count decreased slightly from 24 hours (4.77) to 36 hours (4.43), with statistical significance (p = 0.0010), indicating potential immune modulation over time. RBC levels remained consistent between the two time points (6.06 vs. 6.07), but this change was statistically significant (p = 0.0015), indicating minimal variations in erythropoietic response. Hemoglobin (HGB) levels showed a slight reduction at 36 hours (12.47) compared to 24 hours (13.24), with statistical significance (p= 0.2378), potentially reflecting delayed effects of venom or treatments. Hematocrit (HCT) decreased modestly from 39.93 at 24 hours to 38.00 at 36 hours, also statistically significant (p =0.0300), aligning with the hemoglobin trend. Platelet (PLT) counts declined slightly, with statistical significance (p= 0.6553), suggesting ongoing thrombocytopenia or delayed responses to treatment.

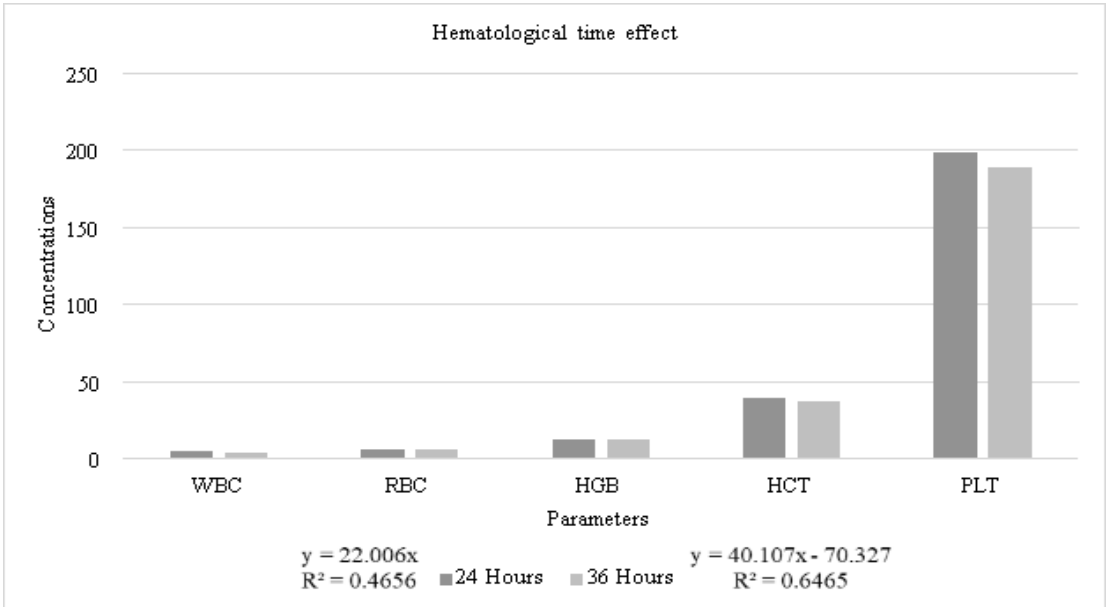

Figure 5: Hematological changes with time

The linear regression equation y = 40.107x - 70.327 indicates that as time and dosing of neutralization increases, the toxic effect decreases, demonstrating a time-dependent neutralization. The coefficient of determination (R^2 = 0.6465) suggests a moderate correlation in 36 hours compared to weaker correlation (R^2 = 0.4656) in 24 hours illustrated in Figure 4. Therefore, as the dose of antivenom and timing increases the effect of the neutralization on envenomation improves.

DISCUSSION

Snakebite envenomations have been ranked by WHO as a neglected tropical disease, causing significant pathological effects, particularly in rural areas of Sub-Saharan Africa (Salvador *et al.*, 2024; Weekers *et al*., 2024). Immunotherapy is the only approved specific treatment against snake toxins, and the production of therapeutic antivenoms requires stringent quality control tests to determine their neutralizing potency (Bala *et al*., 2023; Du *et al.*, 2024).

The venom pool used for animal vaccination, the manufacturing host, and the antivenom purification process and quality control are key factors influencing the neutralization potential and immunogenicity of antivenoms. Enhancing the quality and production capacity of antivenom is a critical step in the WHO's 2021 strategy against snakebite envenomation (Rathore *et al.*, 2023).

Snakebites, particularly involving Echis ocellatus, pose a significant medical emergency in the savannah regions of West Africa. These bites lead to hemorrhage and coagulopathy due to the high contents of snake

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venom metalloproteinases (SVMPs), phospholipase A2 (PLA2s), and serine proteases (SVSPs) in the venom, resulting in high morbidity (Ros-Lucas *et al.,* 2022; Tianyi *et al.,* 2023).

The protein concentration of the partially purified *Echis ocellatus* antibody derived from *Echis ocellatus venom* was calculated using the equation extrapolated from the standard protein curve (Zhang *et al.,* 2023). Our findings, which align with Tola and Missihoun, (2023), shows variability in the protein concentrations between whole serum A (10.06 mg/mL) and whole serum B (4.20 mg/mL). This variability can be attributed to differences in sample preparation, purification efficiency, or inherent biological variability in the antibody yield. Variations in protein concentrations likely result from differences in the rabbits' immune responses, influenced by genetics, health, and metabolism (Duffy, 2020; Corcoran and Karlsson Hedestam, 2024). Whole serum A demonstrated a stronger immune reaction, yielding higher protein levels (Rajabi *et al.*, 2022; Moorlag *et al*., 2024). Despite receiving the same dose under identical conditions, the rabbits likely processed the venom differently (Vergis *et al.,* 2021; Garwolińska *et al.,* 2023). The efficient detoxification and stronger immune response in rabbits from whole serum A led to higher protein production, while those from whole serum B had a weaker response, resulting in a lower yield (Demšar Luzar *et al*., 2021).

Differences in the partial purification process could also explain the variation in protein concentrations (Allaband *et al.,* 2024). Inconsistencies in sample collection or purification methods, such as centrifugation or chromatography, may have affected protein recovery and measurement accuracy (González-Domínguez *et al.,* 2020).

The SDS-PAGE analysis provides a clear differentiation between the antibody and venom samples, confirming their molecular compositions and integrity. The antibody samples (A1, A2, A4) displayed a strong band at approximately 150kb, which corresponds to intact IgG antibodies, with the intensity varying based on the antibody-to-buffer ratio. The strongest band in A2 (20:20 ratio) suggests an optimal antibody concentration, while weaker bands in A1 and A4 indicate dilution effects. Additionally, it aligns with Tijani *et al*. (2024) that the faint bands at ~50kb and ~25kb suggest partial degradation or fragmentation of IgG into its heavy and light chains, a common occurrence during purification or storage (Manson *et al*., 2022; Kpordze *et al*., 2024).

In contrast, the venom samples (V1, V2) exhibited multiple distinct bands at ~85kb**,** ~45kb, and ~25kb, reflecting the presence of venom proteins such as metalloproteinases, serine proteinases, and phospholipases, which are responsible for venom toxicity, including hemotoxic and cytotoxic effects (Machado Braga *et al*., 2020; Schluga *et al*., 2024). These findings validate the antibody preparation, demonstrating its structural integrity while also characterizing the venom protein composition (Lee *et al*., 2021). The results serve as a foundation for further functional assays, such as neutralization studies, to assess the efficacy of the antibodies against venom toxicity. This analysis also highlights the importance of optimizing antibody purification and storage conditions to minimize fragmentation while ensuring maximum potency for therapeutic applications (Lian *et al.,* 2022).

The lethality of snake venom varies significantly based on the geographical origin of the snake, as reported by Hia *et al*. (2020). They found that the intravenous median lethal doses (LD_{50}) in mice ranged from 0.45 to 2.55 mg/kg. In this study, the LD_{50} of *Echis ocellatus* venom was determined to be 0.77 mg/kg, based on the dose response from the laboratory findings. Previous studies by Ajisebiola *et al.* (2024) and Adeyi *et al.* (2021) recorded LD_{50} values of 0.22 mg/kg in Osun State, while Tijani *et al*. (2024) reported 0.35 mg/kg in Borno State, Nigeria. Abd El-Aziz *et al.* (2020) reported an LD_{50} of 1.744 mg/kg for *Echis pyramidum* venom in Egypt, highlighting the influence of geographic and ecological factors on venom potency (Mora-Obando *et al.,* 2023).

For *Echis ocellatus*, regional differences within the same species may be attributed to intraspecific variations in venom composition, influenced by local prey types, environmental pressures, and subtle genetic differences (Tasoulis *et al.,* 2022; Mozhaeva *et al.,* 2024). This could explain why *Echis ocellatus* venom from Northeastern Nigeria (Kano State) exhibited a higher LD_{50} of 0.77 mg/kg compared to 0.35 mg/kg in Northern Nigeria (Borno State) and 0.3 mg/kg in Eastern Nigeria. The significantly higher LD_{50} of *Echis pyramidum* venom from Egypt reflects interspecific differences within the *Echis genus* (Modahl *et al.,* 2020). These

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findings underscore the role of ecological and evolutionary adaptations in shaping venom variability across regions and species (Chaisakul *et al.,* 2020; Almeida *et al.,* 2021).

The LD_{50} value indicates the venom dose that caused mortality in 50% of the test population (mice), providing a quantitative measure of its acute toxicity. Muscle twitching observed in the mice, followed by death within 7-8 hours, reflects the action of specific venom components, such as neurotoxins and mycotoxins (Liang *et al.,* 2020; Huynh *et al.,* 2022). These toxins interfere with neuromuscular signaling, leading to systemic paralysis and respiratory failure, common causes of death in envenomation cases (de Nigris *et al*., 2021).

At lower venom doses, some mice survived, suggesting dose-dependent toxicity. This variability in individual susceptibility could be influenced by factors such as body weight, metabolism, and immune response efficiency (Dornelles *et al.,* 2022). The LD_{50} value provides a baseline for assessing the efficacy of ASV and other neutralizing agents, often evaluated by their ability to protect test animals from lethal doses of *Echis ocellatus* venom (Doering *et al.,* 2020). Understanding the lethal dose and associated physiological effects of *Echis ocellatus* venom is crucial for developing therapeutic interventions. It provides insights into envenomation dynamics, enabling the optimization of antivenom formulations and dosages (Li *et al.,* 2024). These findings also highlight the importance of prompt treatment in clinical settings, as the onset of toxicity is rapid, with death occurring within hours (Dearden and Hewitt, 2021).

The neutralization of *Echis ocellatus* venom at $2LD_{50}$ (double the median lethal dose) by the *Echis ocellatus* antibody produced in rabbits demonstrated its remarkable efficacy, as all animals survived even at the lowest tested concentration (Mozgunov *et al.,* 2022). The effective dose (ED_{50}) for neutralization was determined to be 0.73 mg/kg, indicating that antivenom efficacy should be enhanced to optimize treatment (Wong *et al.,* 2021). There was complete protection of animals at antibody concentrations of 10 mg/kg, highlighting the potency and specificity of the *Echis ocellatus* antibody in neutralizing its venom (Aouni *et al*., 2020). This underscores its potential as a therapeutic tool in managing *Echis ocellatus* envenomation (Röver *et al.,* 2022). The protective effect of the antibody can be attributed to its specificity for the venom's toxic components, likely involving the binding and neutralization of critical toxins such as phospholipases, serine proteases, and metalloproteinases (Bailon Calderon *et al.,* 2020). These venom components are known to cause systemic damage, and their inhibition is vital for survival (Ferreira E Ferreira *et al.,* 2023).

The study demonstrated that the *Echis ocellatus* antibody produced from rabbit immunization effectively neutralized *Echis ocellatus* venom at $2LD_{50}$, with an ED_{50} of 0.73 mg/kg. These findings emphasize its potential as a species-specific, highly effective therapeutic agent for managing envenomation, paving the way for further development and application in high-risk regions (Bowman *et al.,* 2022; Shalabi *et al.,* 2022).

The hematological damage caused by *Echis ocellatus* venom and the protective effects of both the *Echis ocellatus* antibody and standard ASV (Sachetto *et al*., 2022; Noutsos *et al.,* 2022). Exposure to $2LD_{50}$ venom resulted in substantial reductions in WBC, RBC, HGB, HCT, and PLT levels, indicating widespread hematological damage, including immunosuppression, hemolysis, and thrombocytopenia (Leão-Torres *et al.,* 2021; Romero-Giraldo *et al.,* 2022; Abu Baker *et al*., 2022).

Both treatments mitigated these effects but with varying efficacy (Kumar *et al.,* 2024). The *Echis ocellatus* antibody showed superior efficacy in improving RBC, HGB, HCT, and PLT levels, suggesting its strong potential in addressing anemia and thrombocytopenia, especially at higher doses (Abukamar *et al.,* 2022). It also enhanced fibrin formation and improved hemostasis, restoring hemostasis in hypocoagulant conditions by promoting fibrin formation and platelet activation. Conversely, the standard ASV was slightly more effective in restoring WBC counts, particularly at lower doses, indicating a potential advantage in immune recovery (Gerardo *et al.,* 2021). The variations in their mechanisms of action highlight the antibody's targeted effect on hematological toxins compared to the broader, but less specific, neutralization provided by the standard ASV.

The observed dose-dependent responses underscore the need for optimized dosing strategies to maximize therapeutic efficacy. The hematological changes reflect a complex physiological response to envenomation and therapeutic interventions, with both recovery and persistent effects noted (Prado *et al.,* 2024). The significant decrease in WBC count suggests immune modulation (Guo *et al.,* 2020), while the stable RBC count indicates

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minimal impact on erythropoiesis or survival. The decline in hemoglobin and hematocrit levels may indicate delayed hemolysis, hemodilution, or subtle anemia (Ramkumar *et al.*, 2023). Additionally, significant declines in platelet counts are consistent with venom-induced thrombocytopenia and potential endothelial damage (Koter *et al.*, 2023). These findings highlight the areas where platelet recovery is most pronounced by enhancing fibrin formation and improving hemostasis (Lian *et al*., 2022).

Overall, the *Echis ocellatus* antibody demonstrates strong therapeutic potential, particularly for anemia and thrombocytopenia, while the ASV shows promise in immune recovery. These findings offer valuable insights for the clinical management of snakebite envenomation (Nayak *et al.*, 2020).

The hematological changes following *Echis ocellatus* envenomation and subsequent antibody treatment. Over the period from 24 to 36 hours, there was a decrease in WBC count from 4.77 to 4.43, suggesting potential immune modulation and continued recovery or stabilization (Azevedo *et al.*, 2020; Lopes-Ferreira *et al*., 2021). Hemoglobin (HGB) levels showed a slight reduction at 36 hours (12.47) compared to 24 hours (13.24), potentially reflecting delayed effects of the venom or treatments. Hematocrit (HCT) decreased modestly from 39.93 at 24 hours to 38.00 at 36 hours, aligning with the hemoglobin trend. Platelet (PLT) counts also declined slightly, indicating ongoing thrombocytopenia or delayed responses to treatment, all statistically significant ($p=3.17\times10^{-11}$) (Lopes-Ferreira *et al*., 2021).

RBC levels remained consistent between the two time points, with a negligible difference in means (6.06 vs. 6.07) but statistically significant (p=0.0015), indicating minimal variations in erythropoietic response (Casimir *et al.*, 2023). These trends underscore the complex interplay between venom effects and recovery processes, emphasizing the need for prolonged monitoring (Ryan *et al.*, 2021).

It could be concluded that there was superior neutralization capacity and improved hematological recovery; supporting the potential application of monospecific antibodies as an advanced therapeutic strategy for snakebite treatment. The species-specific antibody could revolutionize snakebite management by offering a more precise and reliable treatment alternative, ultimately reducing mortality and long-term complications associated with *Echis ocellatus* envenomation.

SUMMARY OF MAJOR FINDINGS

i. Venom Lethality: The LD_{50} of *Echis ocellatus* venom was 0.77 mg/kg, with regional variations influencing toxicity.

ii. Antibody Characterization: SDS-PAGE analysis confirmed intact IgG antibodies (~150 kDa) with minimal degradation.

iii. Neutralization Efficacy: *Echis ocellatus* antibody successfully neutralized venom at $2LD_{50}$, with an ED_{50} of 0.73 mg/kg and complete protection at 10 mg/kg.

iv. Hematological Effects: Venom exposure caused significant reductions in WBC, RBC, HGB, HCT, and PLT levels, indicative of systemic toxicity.

v. Therapeutic Comparison: The *Echis ocellatus* antibody demonstrated superior efficacy in restoring RBC, HGB, and PLT levels, while ASV showed a stronger impact on WBC recovery.

vi. Clinical Implications**:** Findings support the development of species-specific antibodies as a promising therapeutic strategy for *Echis ocellatus* envenomation.

CONCLUSIONS

This study confirms the effectiveness of *Echis ocellatus* antibodies in neutralizing venom toxicity, emphasizing their relevance in snakebite treatment. Variability in antibody protein concentrations highlights the impact of immune response differences, purification techniques, and storage conditions on antivenom potency. SDS-PAGE analysis verified antibody integrity, supporting their therapeutic potential. The LD_{50} of *Echis ocellatus* venom varied by region, underscoring the need for localized antivenom production.

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Hematological assessments proved crucial in evaluating envenomation effects and treatment success. The *Echis ocellatus* antibody provided superior protection against venom-induced anemia and thrombocytopenia, reinforcing its role as a promising targeted therapy.

RECOMMENDATIONS

a. Optimization of Antibody Production: The variability in antibody yield suggests a need for standardized immunization protocols, purification methods, and quality control measures to enhance consistency and potency.

b. Further Functional and Neutralization Studies: Additional in vivo and in vitro assays should be conducted to assess long-term efficacy, toxicity, and dosage optimization of the *Echis ocellatus*-specific antibody.

c. Regional Venom Characterization: Since venom composition varies geographically, region-specific venom profiling should be integrated into antivenom development to improve therapeutic outcomes.

d. Comparative Clinical Trials: The efficacy of species-specific antibodies versus standard ASV should be evaluated in clinical settings to validate their therapeutic superiority and guide treatment protocols.

e. Development of Combination Therapy: Given the differential hematological effects, a combined approach using both species-specific antibodies and standard ASV may offer enhanced protection against envenomation.

f. Policy and Capacity Building: Strengthening local antivenom production facilities and establishing regulatory frameworks can ensure the availability of high-quality therapeutic antibodies, particularly in endemic regions.

Conflict of Interest

There is no conflict of interest in the course of the research.

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I am a Medical Biochemist, Medical Laboratory Scientist, and Doctor of Philosophy Medical Laboratory Sciences (in view) specializing in Chemical Pathology. My professional journey has been characterized by a commitment to advancing medical research, laboratory diagnostics, and therapeutic strategies, particularly concerning infectious diseases like HIV and snake bite diagnostics.

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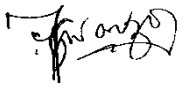
2. DFID on Snakebite with Prof Abdulrazaq Habib Garba as a Lead

3. DFID on monoclonal antibodies as antivenom with Dr Davies USA as Lead



Prof Muhammad Yalwa Gwarzo PhD FWACMLS

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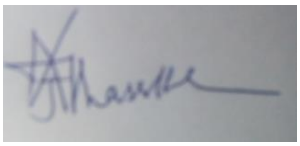
I have over twenty-two years of experience as a university academic staff and a researcher in areas relating to Clinical, Food and Nutrition, Environmental Biochemistry and Enzymology. Holds a Doctorate degree in Biochemistry (Clinical and Nutritional Biochemistry) after successful completion of a thesis entitled “Medicinal and Nutritional properties of white Grubs” from Bayero University, Kano. This was preceded by an MSc degree in Biochemistry (Thesis titled “levels of Nutritional and Non-nutritional Mineral Elements in Water Sediment and Fish of *Kwalkwalawa* River Sokoto Nigeria) and BSc. Degree in Biochemistry (project titled: Incidence of Phenylketonuria Among Mentally Reradiated Children in Torri Home Kano Nigeria). One year Sabbatical to Northwest University, Kano (2013 - 2014). I supervised and graduated over one hundred (100) BSc Biochemistry and BSc Nutrition and Dietetics, over twenty (30) MSc Biochemistry, MSc Nutrition, MSc. Biotechnology, MSc. Public Health Nutrition and Policy students, MMLS and eight (10) PhDs and PMLs. I’m a registered member of Nigerian Society of Biochemistry and Molecular Biology (NSBMB) and National Institute of Food Science and Technology (NIFST). Member and assistant coordinator Northwest in a committee for the compilation of Nigerian Food Composition Table.



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