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Efficacy Testing of Commercially Available Anti-snake Venoms against *Echis ocellatus* Venom in Northern Nigeria

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Abstract

Snakebites cause considerable morbidity and mortality worldwide, with the highest burden found in Sub-Saharan Africa and South Asia. The aim of this study was to evaluate the efficacy of Echitab-Plus-ICP and Premium Antisnake venoms (ASVs) against Echis ocellatus snake venom. The LD_{50} of the venom in experimental mice was determined using probit analysis. The anti-lethality study of the Echitab Plus-ICP ASV at doses (3, 6, 9, 12 and 15 mL/kg) and Premium ASV at doses (0.1, 0.2, 0.3, 0.4, and 0.5 mL/kg) was carried out according to Theakston and Reid method. Three doses (0.4, 0.8, and 1.2 mL/kg) of the venom were administered intramuscularly (i.m) and the Minimum Hemorrhagic Dose (MHD) was determined. The venom's LD_{50} and MHD in experimental mice were 4 mg/kg and 0.4 mg/kg respectively. The Echitab Plus-ICP ASV at doses (3, 6, 9, and 15 mL/kg) and the Premium ASV at the doses (0.1, 0.2, 0.3, and 0.4 mL/kg) produced 100% protection against lethality induced by the $2LD_{50}$ of the Echis ocellatus snake venom. However, Echitab Plus-ICP ASV at a dose of 12 mL/kg and Premium ASV at the dose of 0.5 mL/kg produced 83% and 50% protection respectively. Furthermore, Echitab-Plus-ICP and Premium ASVs at all doses produced statistically significant ($p<0.05$) reduction in hemorrhage-induced by 2MHD of the venom. Also, an in-vitro hemolysis assay of the two ASVs showed significant ($p<0.05$) reduction in venom-induced red blood cells hemolysis. These findings suggest that Echitab-Plus-ICP and Premium Anti-venoms are effective against

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envenomings caused by Echis ocellatus venom. Further experiments should be conducted on these ASVs so as to study its necrotizing, myotoxic and pro-coagulant properties.

Keywords: Snakebite**,** Venom, Anti-venom, *Echis ocellatus*, Hemolysis, Coagulation.

INTRODUCTION

Snakebite envenoming is a neglected tropical disease with a high burden of morbidity and mortality, especially in deprived rural areas of Africa, Asia, Latin America and parts of Oceania (Gutiérrez *et al*., 2009; Gutiérrez *et al*., 2013; Harrison & Gutiérrez, 2016). The World Health Organization (WHO, 2016) has recognised snakebite as a category A neglected tropical disease. Snakebite is increasing in northern Nigeria and is becoming an important occupational and public health hazard (Michael *et al*., 2018; Habib *et al*., 2020; Bala *et al*., 2021). Snakebites by the viperid or *Echis ocellatus* snakes are very frequent in the savanna region of West Africa (Abubakar *et al*., 2010a; Habib, 2013). *Echis ocellatus* is one of the most medically important snakes in northern Nigeria accounting for about 95% of the recorded snakebite cases (Yusuf *et al*., 2015; Adeyi *et al*., 2021) which occurs mostly during farming or livestock herding (Habib *et al*., 2015; Habib *et al*., 2020; Bolon *et al*., 2021).

Echis ocellatus produces on average about 18 mg of dry venom, with a recorded maximum of 72 mg as it may inject as much as 12 mg, whereas the lethal dose for an adult is estimated to be only 5 mg (Mirtschin *et al*., 2017; Adeyi *et al*., 2022). The venom is rich in serine proteinases and metalloproteinases, which affect the blood coagulation, platelet aggregation, blood pressure, nervous system, and complementary system (Wagstaff & Harrison, 2006). One of the most prominent effects of the snake venom of *E. ocellatus* is coagulation activity that it employs in killing its preys (Xie *et al*., 2020; Salmanizadeh *et al*., 2013). Snake venoms affect hemostasis by activating or inhibiting coagulant factors or platelets, or by disrupting endothelium (Clemetson *et al*., 2007). The venom from this species is used in the manufacture of several drugs, examples is Echitastin. Echistatin is a single-chain polypeptide with a molecular weight of 5400 and a native isoelectric point of 8.30. Echistatin contains the sequence arginine-glycine-aspartic acid, which is common to proteins which bind to the glycoprotein IIb/IIIa complex coagulation factor. It also contains the sequence proline arginine-asparagine-proline, which is found in the A-alpha chain of human fibrinogen at position 267-270 (Gan *et al*., 1988; Benjamin *et al*., 2011). The purified protein inhibits fibrinogen-dependent platelet aggregation initiated by ADP and also prevents aggregation initiated by thrombin, epinephrine, collagen, or platelet activating factor (Gan *et al*., 1988; Garsky *et al*., 1989). Venom proteins affect platelet function by binding or degrading Von Willebrand Factor (VWF) or platelet receptors, activating protease-activated receptors or modulating Adenosine Diphosphate (ADP) release and thromboxane A_2 formation (Lu *et al*., 2005; De Queiroz *et al*., 2017). Some venom enzymes cleave key basement membrane components and directly affect capillary blood vessels and cause haemorrhage (Lu *et al*., 2005). Envenomation results in local symptoms as well as severe systemic symptoms that may prove fatal. Local symptoms include swelling and pain, which appear within minutes of a bite (Adukauskienė *et al*., 2011). In very serious cases, swelling may extend up the entire affected limb within 12-24 hours and blisters form on the skin (Mehta & Sashindran, 2002; Weinstein *et al.,* 2009; Tednes & Slesinger, 2022). The venom yield from individual specimens varies considerably, as does the quantity injected per bite (Ali *et al*., 2004; (Mirtschin *et al*., 2017). The mortality rate from snakebite is about 20%, and currently deaths are very rare because of the availability of anti-venom (Ali *et al*., 2004; Ainsworth *et al.,* 2018; Kontsiotis *et al.,* 2022). Severe systemic symptoms of snakebite include hemorrhage and coagulation defects and

haematemesis. Others include melena, haemoptysis, haematuria and epistaxis which may lead to hypovolemic shock (Ali *et al*., 2004). Furthermore, majority of the patients of snakebite develop oliguria or anuria within a few hours to 6 days post bite. In some cases, acute renal failure (ARF) may result which could warrant renal dialysis (Levi & Cate, 1999; Ali *et al.,* 2004; Kontsiotis *et al.,* 2022).

Antisnake venoms (ASVs) are produced by fractionating plasma obtained from horses previously hyper-immunized with the relevant venoms. Antisnake venoms may be monovalent or polyvalent (WHO, 2016). In antisnake venom, multiple factors such as immunoglobulin G (IgG) concentration, titre, avidity and protein specificity play a crucial role in determining its efficacy (Williams & Chase, 2014). The above factors may be affected by the quality of the immunizing venoms, the quantitative ratio used, the selected adjuvants and the manufacturing protocols (León *et al*., 2014; Harrison *et al.,* 2017). Thus, it is very difficult to implicate a single aspect as being primarily responsible for the lack of efficacy of antivenom (Harrison *et al*., 2017).

Increasing cost and scarcity of anti-snake venoms provide an opportunity for proliferation and unscrupulous marketing of geographically-inappropriate anti-snake venoms that have proved clinically disastrous (Abubakar *et al*., 2010b; Bala *et al*. 2021), ineffective or fake in the region (Yusuf *et al*., 2015). Bala *et al*. (2021) reported that northern Nigeria has the highest snakebite cases in Nigeria and if urgent measures are not taken immediately, the incidences would continue to worsen. This necessitates the screening of the available anti-snake venoms in the region to identify candidate anti-snake venoms with specific activity and therefore effective against the venom of *Echis ocellatus* (saw-scaled or carpet viper) in the region (northern Nigeria). This will go a long way to help in improving the quality of life to snakebite victims and to increase the confidence among the clinicians in choosing appropriate anti-snake venom.

MATERIALS AND METHODS

Source and Maintenance of Experimental Mice

One hundred and seventy-four (174) Swiss Albino mice of both sexes weighing (18 – 20 g) were purchased from the Department of Pharmacology and Therapeutics, Bayero University, Kano. They were maintained according to the standard guidelines for the use of laboratory animals. The animals were kept at room temperature (25 ^{0}C) and 12-hours light/12-hours dark circle. The relative humidity was maintained between 50 - 60%. The animals were fed on Vital Feed (Bukuru, Jos) and water *ad libitum*. The experiment was approved by the Ethical Committee, College of Health Sciences Bayero University, Kano, Nigeria. Ref No: BUK/CHS/HREC/VII/66.

Source and Maintenance of Experimental Snake

Four (4) *Echis ocellatus* (Carpet viper) were obtained from Duguri Village, Alkaleri Local Government Area of Bauchi State. They were kept and maintained at the Serpentarium of the Department of Pharmacology and Therapeutics, Bayero University, Kano. The snakes were identified by Yusuf Peter Ofemele of the Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria.

Source of Antivenom
The two most commonly available ASVs selected are commercially available in northern Nigeria including; Echitab-Plus-ICP and Premium were Purchased from a standard community pharmacy store in Gombe state.

Venom Milking and Preparation
The venom was milked according to the method described by Bala *et al*., (2022). The pooled venom was placed in a desiccator containing activated silica and allowed to dry at room temperature. The crystallized venom was subsequently transferred into a refrigerator and stored at 4 ^{0}C.

Determination of LD_{50}
Thirty (30) Swiss Albino mice weighing 18 to 20 g were randomly selected and divided into five (5) groups of six (6) mice each. Groups I, II, III, IV and V received 0.5, 1, 2, 4 and 8mg/kg of the venom i.p respectively. The number of the mice that died and the time of death were recorded within 48 hours of venom administration. The dose of the venom that killed 50% of the mice (LD_{50}) within 48 hours was determined using Probit's Analysis (Theakston & Reid, 1983).

Anti-lethality Assay
Sixty-six (66) mice were divided into eleven (11) groups of six (6) mice each. Group I was given a challenge dose of the venom ($2LD_{50}$) i.p. Groups II - VI were treated with five graded doses (3, 6, 9, 12 and 15 mL/kg) of the premixed Echitab-Plus-ICP ASV and $2LD_{50}$ of the venom, and incubated for 30 minutes at 37 °C. Groups VII - XI were treated with five graded doses (0.1, 0.2, 0.3, 0.4, and 0.5 mL/kg) of the premixed Premium ASV and $2LD_{50}$ of the venom, and incubated for 30 minutes at 37 °C. The rate of mortality was recorded in each group within 48 hours of administration of the mixture of venom plus antivenom (Theakston & Reid, 1983).

Determination of Minimum Hemorrhagic Dose
Twelve (12) mice weighing (18-20 g) were divided into three (3) groups of four (4) mice each. They were injected intradermally with three (3) graded doses (0.4, 0.8 and 1.2 mg/kg) of the venom dissolved in distilled water. Three (3) hours later, the mice were sacrificed and their skins removed, and then the hemorrhagic area in the inner side of the skin was measured using Vernier caliper (Theakston & Reid, 1983). The minimum hemorrhagic dose MHD is the amount of venom that induces a hemorrhagic area of 10 mm diameter within 3 hours of venom administration (Gutiérrez *et al*., 1985). The MHD was determined by plotting mean lesion diameter against venom dose (Theakston, 1986).

Venom-Induced Hemorrhage Neutralization Assay
Sixty-six (66) mice were divided into eleven (11) groups of six (6) mice each. Group I was given 2MHD of the venom i.p. Groups II - VI received five graded doses (3, 6, 9, 12 and 15 mL/kg) of the premixed Echitab-Plus-ICP ASV and 2MHD of the venom i.p. Groups VII - XI received five graded doses (0.1, 0.2, 0.3, 0.4, and 0.5 mL/kg) of the premixed Premium ASV plus 2MHD of the venom i.p. Three hours later, the hemorrhagic lesion in the inner side of the skin was measured (Theakston, 1986).

Determination of Minimum Coagulant Concentration
Graded venom concentrations ranging from 0.015, 0.030, 0.060, 0.120 and 0.240 mg/mL each were added to 0.5 mL of rabbit blood at 37 °C in new glass clotting test tubes. The solutions were mixed, and the clotting time was recorded. Minimum anticoagulant concentration

(MAC) is the amount of venom that will prevent blood coagulation for up to 12 minutes (Theakston & Reid, 1983). The MAC was calculated by plotting clotting time against venom concentration. Also, the concentration was determined at 12 minutes of the clotting time (Theakston & Reid, 1983).

Coagulant Activity Neutralization Assay
Mixtures of a challenge dose of venom determined in the dose-finding study above and various dilutions of ASV were prepared to contain the challenge dose of venom in 50 µL of the mixture. The mixtures were incubated at 37 °C for 30 minutes, and aliquots of 50 µL were added to 0.5 mL of whole blood (Theakston & Reid, 1983). The control was incubated with venom solution alone. The formation or absence of clots was observed by tilting the test tube after every one minute, and clotting time was recorded (Benjamin *et al*., 2018).

Anti-hemolytic Activity
Blood samples were drawn from the marginal vein of the rabbit's ear and centrifuged at 2,800 RCF for five minutes. Two percent erythrocyte suspension was prepared in sterile phosphate buffer saline (PBS) (Malagoli, 2007). The mixture of a fixed venom concentration and various dilutions of anti-snake venoms were prepared to contain the challenging dose of venom in 50 µL. Then 300 µL of the 2% suspension erythrocytes was added to the test tubes. The mixture was incubated at 37 ^{0}C for 30 minutes, centrifuged, and the supernatant layer was used to measure the absorbance of the liberated hemoglobin at 540 nm. The percentage of hemolysis was calculated by dividing the sample's absorbance with that of positive control multiplied by 100 (Gould *et al*., 2000).

Hematological Analysis
Blood samples were collected from the eleven (11) groups of mice treated for hemorrhagic studies and analyzed using the Ray to hematology machine (Model: RT7600).

Statistical Analysis
Data obtained was expressed as Mean ± SEM. The differences between means were analyzed using one-way ANOVA by SPSS Version 22. Significant means were separated through Dunnet's post hoc test. $p<0.05$ were considered statistically significant.

RESULTS

Determination of Median Lethal Dose (LD_{50}) of *Echis ocellatus* Venom
The LD_{50} of the *Echis ocellatus* venom was estimated at 4.0 mg/kg.

Effect of Echitab-Plus-ICP and Premium Antivenoms on *E. ocellatus* Venom Induced Lethality on Experimental Mice.
During the experiment, all the animals in the control group that received $2LD_{50}$ (8 mg/kg) of the venom alone died (100% mortality). The Echitab-Plus-ICP ASV at the doses of 3, 6, 9, 15 mL/kg produced 100% protection against mortality induced by the $2LD_{50}$ of the *E. ocellatus* venom compared to the control group. Also, Premium ASV at the doses of 0.1, 0.2, 0.3 and 0.4 mL/kg produced 100% protection against mortality induced by the $2LD_{50}$ of the *E. ocellatus* venom. However, Echitab-Plus-ICP ASV at the dose of 12 mL/kg produced 83% protection, and Premium ASV at a dose of 0.5 mL/kg produced 50% protection against mortality induced by the $2LD_{50}$ of the *E. ocellatus* venom compared control group (Table 1).

Table 1: Effect of Echitab-Plus-ICP and Premium antivenom on $2LD_{50}$ *E. ocellatus* venom-induced lethality on mice. n=6.

Treatment groups (ml/kg) + $2LD_{50}$ venom		Mean Latent Onset of Death (min)	Mortality (n=6)	(%) Protection
Venom	8.0	72.0 ± 12.01	6/6	0
ECHI TAB	3.0	0.0 ± 0.0	0/6	100
ECHI TAB	6.0	0.0 ± 0.0	0/6	100
ECHI TAB	9.0	0.0 ± 0.0	0/6	100
ECHI TAB	12.0	160.0 ± 0.0	1/6	83
ECHI TAB	15.0	0.0 ± 0.0	0/6	100
PAV	0.1	0.0 ± 0.0	0/6	100
PAV	0.2	0.0 ± 0.0	0/6	100
PAV	0.3	0.0 ± 0.0	0/6	100
PAV	0.4	0.0 ± 0.0	0/6	100
PAV	0.5	153.0 ± 17.64	3/6	50

Data is presented as Mean ± S.E.M., * = p<0.05, ** = p<0.01 compared to venom group. ECHI TAB = Echitab-Plus-ICP Antivenum, PAV = Premium Antivenom.

Minimum Hemorrhagic Dose (MHD) of *Echis ocellatus* venom

The Minimum Hemorrhagic Dose (MHD) of *E. ocellatus* venom in mice was 0.4 mg/kg which is the dose at 10.0 mm hemorrhagic diameter as stated in Theakson & Reid, (1983) (Table 2).

Table 2: Minimum Haemorrhagic Dose (MHD) of *Echis ocellatus* venom. n=3.

Group	Dose (mg/kg)	Mean Hemorrhagic Length (mm)
I	1.2	19.5 ± 1.88
II	0.8	15.5 ± 3.17
III	0.4	10.0 ± 0.73

Data is presented as Mean ± S.E.M., * = p<0.05, ** = p<0.01 compared to venom group.

Effects of Echitab-Plus-ICP and Premium ASV on 2MHD *E. ocellatus* venom induced-hemorrhage

In this experiment, the control group at dose of 2MHD produced a hemorrhagic diameter of 27.00 ± 1.81 mm. The Echitab-Plus-ICP produced statistically significant (p<0.05) reduction in the *E. ocellatus* venom-induced hemorrhage at all doses compared with the control group. Also, premium antivenoms produced statistically significant (p<0.05) reduction in the *E. ocellatus* venom-induced hemorrhage at all doses compared with the control group (Table 3).

Table 3: Effect of Echitab-Plus-ICP and Premium Antivenom ASVs on (*Echis ocellatus*) venom-induced Hemorrhagic lesion in Mice, n=6.

Treatment (ml/kg) + 2MHD Venom		MHL (mm)	QPMHD	(%) PMHD
Venom	0.8	27.00 ± 1.81	0/6	0.00
ECHI TAB	0.3	5.00 ± 0.58*	3/6	50.00
ECHI TAB	0.6	5.00 ± 1.00*	3/6	50.00
ECHI TAB	0.9	6.00 ± 0.00*	4/6	66.67
ECHI TAB	1.2	2.50 ± 0.50*	4/6	66.67
ECHI TAB	1.5	2.67 ± 0.33*	3/6	50.00
PAV	0.01	4.50 ± 1.50*	4/6	66.67
PAV	0.02	0.00 ± 0.00	6/6	100.00
PAV	0.03	0.00 ± 0.00	6/6	100.00
PAV	0.04	0.00 ± 0.00	6/6	100.00
PAV	0.05	0.00 ± 0.00	6/6	100.00

Data is presented as Mean ± S.E.M., * = p<0.05, ** = p<0.01 compared to venom group. ECHI TAB = Echitab-Plus-ICP Antivenum, PAV = Premium Antivenom

Effects of Echitab-Plus-ICP and Premium ASVs on *E. ocellatus* venom-induced Anticoagulation

The minimum anticoagulant dose of the *E. ocellatus* venom was 0.2 mg/mL which is the concentration of the venom determined at 12 minutes of the clotting time using rabbit erythrocytes (Table 4).

Table 4: Minimum Anticoagulant Concentration (MAC)

Venom (mg/ml)	Time (min)
0.015	3.48
0.030	5.33
0.060	6.92
0.120	8.20
0.240	13.60

Data is presented as Mean ± S.E.M., * = $p<0.05$, ** = $p<0.01$ compared to venom group.

Effects of Echitab-Plus-ICP and Premium ASV on *E. ocellatus* venom-induced hemolysis in rabbit erythrocytes

The results showed that Echitab-Plus-ICP ASV at concentration of 70 µL produced 53% protection, at 140 µL produced 68% protection and at 280 µL produced 73% protection in concentration dependent manner compared to the control group. In addition, Premium Antivenom at concentration of 2 µL produced 7% protection, at 4 µL produced 26% protection and at 8 µL produced 36% protection in concentration dependent manner. Therefore, Echitab-Plus-ICP at all concentration offered better protection against *E. ocellatus* venom-induced hemolysis than the Premium antivenom (Table 5).

Table 5: Inhibitory Effect of two different ASVs on *E. ocellatus* venom-induced hemolysis in rabbit erythrocytes

Treatment (µL)		Absorbance	% Hemolysis	% Protection against Hemolysis
Venom (mg/ml)	0.2	4.178	100	0
ECHI TAB	70	1.960	47	53
ECHI TAB	140	1.338	32	68
ECHI TAB	280	1.116	27	73
PAV	2	3.901	93	7
PAV	4	3.095	74	26
PAV	8	2.0708	64	36

Data is presented as Mean ± S.E.M., * = $p<0.05$, ** = $p<0.01$ compared to venom group. ECHI TAB = Echitab-Plus-ICP Antivenum, PAV = Premium Antivenom.

Effect of Echitab-Plus-ICP and Premium ASVs on hematological parameters of mice injected with *E. ocellatus* venom

In this experiment, both Echitab-Plus-ICP and Premium ASVs did not produce any statistically significant ($p>0.05$) changes in all the hematological parameters except a significant ($p<0.05$) increase in platelet compared to the control group (Table. 6).

Table: 6 Effect of Echitab-Plus-ICP and Premium Antivenom on hematological parameters of mice injected with *Echis ocellatus* venom, (n=6).

Treatment groups (ml/kg)+ 2MHD Venom		PLT (×10³ µL)	WBC (×10³ µL)	RBC (×10³ µL)	HGB (g/dL)
Venom	0.8	132.17±13.36	4.47±0.30	4.88±0.32	15.12±0.82
ECHI TAB	0.3	238.80±30.94*	5.02±0.37	4.90±0.29	14.53±1.02
ECHI TAB	0.6	253.80±19.02*	5.58±0.53	4.82±0.80	14.72±0.59
ECHI TAB	0.9	229.00±32.41*	5.47±0.56	4.92±0.13	14.18±0.36

ECHI TAB	1.2	286.77±28.97**	5.82±0.77	4.72±0.11	13.50±1.15
ECHI TAB	1.5	263.50±39.11*	5.12±0.21	4.92±0.24	13.75±0.59
PAV	0.01	236.77±43.03*	5.95±0.62	5.12±0.19	21.98±8.28
PAV	0.02	268.17±34.29*	4.57±0.26	4.76±0.14	13.87±0.36
PAV	0.03	344.83±16.93**	5.28±0.36	4.73±0.22	14.48±0.92
PAV	0.04	214.00±32.15*	5.08±0.42	5.13±0.47	12.11±1.24
PAV	0.05	292.60±34.28**	5.40±0.58	5.07±0.22	12.70±0.85

Data is presented as Mean ± S.E.M., * = $p<0.05$, ** = $p<0.01$ compared to venom group. PLT = Platelets, WBC= White blood cells, RBC=Red blood cells, HGB = Haemoglobin ECHI TAB = Echitab-Plus-ICP Antivenum, PAV = Premium Antivenom

DISCUSSION

During the course of this study, a considerably high median LD_{50} (4mg/kg) was obtained from the venom milked from *E. ocellatus*. This implies that the venom recorded in this study is highly toxic (Rekosh, 1977; Theakston & Reid, 1983). This finding is similar to the findings of Yunusa *et al*., (2017) that reported LD_{50} (1.24 mg/kg) coupled with Ernst & Zug, (1996) that reported LD_{50} (0.23 mg/kg) in their respective studies. However, the LD_{50} was much lower than 11.1 mg/kg reported in another study involving *E. ocellatus* (Salmanizadeh *et al*., 2013). The differences in the LD_{50} could be due to differences in geographical locations of the snakes, sex, diet, and seasonal variation. Also, it could be due to differences in the compositions, method of concentration and relative abundance of venom toxins (Chippaux *et al*., 1998).

The ability of anti-snake venoms (ASVs) to prevent mortality induced by the *E. ocellatus* venom was assessed according to the standard method (Theakson & Reid, 1983). Accordingly, the two anti-venoms tested were found to offer a 100% protection against the $2LD_{50}$ of *E. ocellatus* venom-induced lethality. Nonetheless, at relatively higher doses of Echitab-Plus-ICP (12 mL/kg) and Premium ASV (0.5 mL/kg) lower level of protection of 83% and 50% were obtained respectively. These findings suggest that the two ASVs experimented effectively managed *E. ocellatus* envenomation because of their ability to neutralize venom-induced lethality (Gutierrez *et al*., 2017).

The minimum hemorrhagic dose (MHD) of *E. ocellatus* venom in mice obtained from this study was 0.4 mg/kg which is the dose at 10.0 mm hemorrhagic diameter as stated Theakson & Reid, (1983). This result was slightly higher than 0.12 mg/kg reported in another related study documented by Prashar *et al*., (2015). Furthermore, the two ASVs (Echitab-Plus-ICP and Premium Antivenom) significantly ($p<0.01$) reduced haemorrhage induced by the 2MHD of the *E. ocellatus* venom compared to the control group. In general, the Premium Antivenom exhibited greater activity at all doses than Echitab-Plus-ICP ASV against *E. ocellatus* venom induced haemorrhagic lesion.

The efficacy of antivenoms to neutralize toxicity of medically-relevant snake venoms has been demonstrated through meticulous preclinical studies. The gold standard in the preclinical assessment and quality control of antivenoms is the neutralization of venom-induced lethality (Gutiérrez *et al.,* 2017). The minimum anticoagulant concentration obtained from *E. ocellatus* venom was 0.2 mg/mL. It is a standard plasma concentration obtained under the same conditions, recording and clotting time (Xie *et al*., 2020). In each case the MAC was calculated by plotting the clotting time against venom concentration and taking reading at a given clotting time (Theakston and Reid, 1983; Xie *et al*., 2020). Several snake venom proteins with no 'detectable' (known or tested) enzymatic activity inhibit blood coagulation (Kini, 2006). A number of non-enzymatic anticoagulant proteins have been purified and characterized. These proteins inhibit the coagulation process through their direct interaction with a specific coagulation factor and phospholipases A2 (PLA2) (Kini, 2006). Therefore, the presence of

PLA2s is the most likely candidates responsible for anticoagulant effect of *E. ocellatus* venom (Kazandjian *et al*., 2021).

In this study, both Echitab-Plus-ICP and premium ASVs prevented RBC hemolysis in a concentration dependent manner. EchiTAb-plus-ICP is an IgG antivenom with a good antihemolytic activities (Sánchez *et al*., 2017). The Echitab-Plus-ICP at all concentrations offered better protection against *E. ocellatus* venom-induced hemolysis than the premium antivenom. Shehu (2011) reported a similar study in which haemolysis of blood due to venom was reduced from 66% to 27.4% when the venom was incubated with the anti-venom. These findings suggest that the two anti-venoms have anti-hemolytic activity thus capable of blocking the venom-induced haemolysis (Shehu, 2011). As recorded in this study, venom of *E. ocellatus* affected the blood coagulation and platelet aggregation due to both metalloproteases and ecarin, an enzyme that activates prothrombin (Harrison *et al*., 2003). The increase in the blood clotting time recorded in this study is a sign of coagulopathy induced by *E. ocellatus* venom. This may be due to metaloproteinases presence in the *E. ocellatus* venom, which is reported to reduce platelets counts and inhibit conversion of fibrinogen to fibrin thereby causing defibrigention and consequently prolonged bleeding (Ho & Chan, 1986).

The results of various hematological parameters investigated in this study are valuable indices for evaluating the toxic effects and possible internal organ damage caused by of *E. ocellatus* venom in animals (Etim *et al*., 2014). This could be seen as a variation in any of the hematological parameters tested. Results indicated that no significant change in white blood cells, red blood cells and hemoglobin concentrations observed in the group treated with ASVs. Similar results have been reported by others investigators (Emam & Nikzamir 2008; Al-Maliki *et al*., 2015; Slagboom *et al*., 2017). However, in this study, a significant increase in the total platelet count was recorded in all the groups treated with ASVs. This may be due to the ability of the ASVs to prevent damage of the blood vessels, and consumption of clothing factors which may cause ulceration and bleeding. This result is in line with the outcome of related studies that reported a significant increase in the total number of blood platelets (Harrison *et al*., 2003; Al-Sadoon *et al*., 2005; Periayah *et al*., 2017).

CONCLUSION

The results obtained in this study indicate that Echitab-Plus-ICP and Premium Antivenom which are commercially available in northern Nigeria are effective against *E. ocellatus* venom-induced lethality, hemorrhagic lesion, coagulopathy and hemolysis. It can be suggested that a study targeting neutralization of other toxic effects of snake venom such as hemorrhagic, coagulopathic, and hemolytic should also be carried out to further establish and confirm the efficacy of the studied anti-snake venoms.

Authors' Contributions

We declared that this work was conducted by the authors named in this article. Yusuf Abubakar Muhammad conceived the original idea, developed the methods, produced the theory, performed laboratory works and co-wrote the manuscript. Sani Malami developed the techniques and co-supervised the work. Auwal Adamu Bala, Abdullahi Rabiu Abubakar and Shehu Yakubu Magaji performed laboratory works, co-wrote and proof read the manuscript. **Abdullahi Ibrahim Doma,** Suleiman Yunusa Goji, Zainab Gambo Ibrahim and Ibrahim Muhammad performed the laboratory works and proof read the manuscript. Basheer AZ. Chedi gave the supervisory approval and finally revised the manuscript for intellectual content.

CONFLICT OF INTERESTS

The authors declared no competing interest

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