Evaluation of Some Biochemical Parameters Associated With Snake Bite Envenomation in Southern Part of Plateau State, Nigeria

Fwogos, Ishaku Chung[1] and Eldad Nandem Johnson[2]

[1]*Department of Medical Laboratory Science, Plateau State College Of Health Technology, Zawan, Plateau State, Nigeria*
[2]*Department of Pharmacy Technician, Plateau State College Of Health Technology, Zawan, Plateau State, Nigeria*

ABSTRACT: Snakebite envenoming is a neglected tropical disease that predominantly affects improvised rural populations in Africa, Asia, Latin America and parts of Oceanic. It inflicts a heavy load in terms of morbidity and mortality and an unknown number of bitted persons end up with permanent, physical and physiological scars. The aim of the study was to determine some biochemical parameters in snakebite envenomation in southern part of plateau state, Nigeria. A total of 243 subjects were used for the study. They were equally grouped into pre-treatment, post treatment and control groups having 81 subjects each. From each sun groups, serum samples were collected and assayed for liver, Kidney and Electrolytes Parameters. The Liver Function Parameters Examined Include Total Protein (TP), Albumin (ALB), Alkaline Phosphate (ALP), Alanine Amino Transferase (ALT) And Aspartate Aminotransferase (AST). The kidney function parameters such as Serum urea, Creatinine and other electrolytes such as Sodium (Na^+), Potassium (K^+), Chloride (Cl^-) and Bicarbonate (HCO_3^-) were also assayed, they were all subjected to standard laboratory procedure. The data obtained were subjected to Analysis of variance (ANOVA), Fishes Least Significance Difference (F-LSD), Post Hoc test and correlation analysis with the aid of SPSS version 22.0.The results of the test showed that there was a significant difference in Creatinine ($P < 0.05$) level of the control subjects when compared to pre and post – treated subjects. Also, while examining some liver function profile of snake bites subjects and controls, only ALT and AST showed significant difference ($P < 0.05$). No significant difference ($P > 0.05$) was recorded in others. The correlation matrixes of all the variables of participants in pre- and post-snake bite treatment groups were examined. While in pre-snake bite treatment group, the results showed that two pairs of variables were significant ($P < 0.05$). These include Na^+ vs. Cl^-; ALT vest Cl^- with r = -0.386 and -0.436 respectively. In post-snake bite treatment group, the results showed that three pairs of variables were significant ($P < 0.05$). These include ALB vs. K^+; AST vs. Urea and ALP vs. Urea with r = 0.415, 0.541 and 0.382 respectively. These parameters are very important to this study and they are significant in biochemical diagnoses of snake bite envenom patients in southern plateau Nigeria. Hence, this study recommended among others that since snake bite envenoming constitutes an important public health problem in parts of southern plateau, Nigeria, important efforts should be prioritized on research, ant venom availability and accessibility

KEYWORDS: Snakebite, envenomation, Total Protein, Albumin, Alkaline Phosphate, Alanine Amino Transferase

I. INTRODUCTION

Snakebite envenoming is a neglected tropical disease that predominantly affects improvised rural populations in Africa, Asia, Latin America and parts of oceanic [21]. It inflicts a heavy load in terms of morbidity and mortality, and an unknown number of bitted persons end up with permanent, physical, physiological and psychological squeale[21].

Research based on hospital statistics indicate that on a worldwide basis, between 1.2 and 5.5 million people are bitten by snakes every year causing 25 000 to 125 000 deaths and leaving an

estimated 400 000 people with permanent biochemical and haematologicalsquelae. [4].

However, these figures underestimate the actual magnitude of this disease, as revealed by recent community-based surveys on incidence and mortality associated with snakes bite envenoming in various countries [11].A growing international awareness in the last decade, as shown by various initiatives aimed at understanding the biochemical and haematological consequences of snakebite and improving prevention and treatment World Health Organization [20].

The venomous snakes in Africa are known to belong to four main families namely; the Colubridea, Elapidea, Viperdea and hydrophidae[19].The elapid and particularly the viperid snakes are responsible for most bites in the savannah region of Africa.

The problem of snakebite is enormous in some parts of Nigeria particularly in Plateau, Gombe and Taraba states. The areas of highest concern are around the South, South east and Northern parts of these states, where they have contiguous borders[9].

The vegetation is typical savannah and the terrain is mountainous and rock, a habitate that is well adapted to E. Carinatus[6].In Nigeria, the most common poisonous snakes are the elapids and the vipers. These include the Najanigricollis (spitting cobra) and the Najamelanolema (black cobra), and the Viperidechiscarinatus (carpet viper) and arietans (puff adder) [1].

However, studies in plateau state of Nigeria have shown that snakebites are mainly caused by two species of snakes namely cobra (Najanigricollis) and carpet viper (echiscarnatus) [1].and majority of the snakes found in the agricultural areas of the states are harmful. The most prevalent venomous snakes in the plateau state of Nigeria are Echiscarinatusocellayus (the saw scaled carpet viper), Bitisarietans (puff adder), and Najanigricollis (the spitting cobra). Other less important snakes include Dipholidus typhus (Boomslang), Causes maculates (Night Adder) and Najahaje (Egyptian cobra) [12].

Snakes venom is considered to be one of the most highly developed and complicated of all toxins produced by plants and animals (Bomb et al., 1996). It is a complex mixture containing polypeptides, enzymes, glycoprotein and other substances capable of several pharmacological activities [13].The more lethal venom fraction of snakes appears to be certain non- enzymatic proteins. In addition, snakes venoms contain an organic substance including metals like iron [13].Some snake venom, contains carbohydrates (glycoprotein) [14].lipids and biogenic amines while other venoms contain free amino acids.

Snake venom consists of at least 26 enzymes although no single venom contains all of these enzymes. At least ten (10) enzymes are found in most of the venoms, while the remaining are scattered throughout the venoms of the five families of poisonous snakes. Elapid venoms are rich in acetylcholinesterase, while Cortaid and viper venoms lack this enzyme but rich in endopeptidase. [12].

The important enzyme in snake venom include: proteolysis enzymes, thrombin like enzymes, argentine ester hydrolase, collagenase, hyaluronidase, phospholipase A, phospholipase B, phospholipase C, lactate dehydroganase, acetyl cholinesterase, RNase, DNase, 5-nucleotidase, phosphomonoesterase[9].

Snake venom mainly affects the cardiovascular, nervous, renal and respiratory systems [8].Due to the high concentration of neurotoxins in the venom of elapid family, the usual chemical manifestations are neuromuscular paralysis, ptosis, opthalmoplegia and bulbar paralysis. On the other hand, the viper venom produces shock, haemorrhage and disseminated intravascular coagulation. [10].

SIGNIFICANCE OF STUDY
Snake venom is considered to be one of the most highly developed and complicated of all toxins produced by plants and animals. The biochemical and haematological changes in an envenom patients are rarely evaluated in the laboratory and these appear too little evidence in literature in this regard especially in tropical and sub-tropical countries in which Nigeria is one of them. The study of biochemical and haematological changes in envenom patients may help to know if increase levels of these parameters may be the etiological causes of certain diseases and organs failure in an envenom patient. There have been inconsistent reports in literature as to the levels of snake venom and the ability to cause biochemical and haematological changes in the system. This study was therefore designed to evaluate biochemical and haematological changes that occur in snake bite envenom patents in southern parts of plateau state, Nigeria.
SCOPE OF STUDY
To assay the levels of biochemical and haematological parameters such as blood urea, creatinine, alkaline phosphatase, alanine transaminases, aspartate transaminases, total protein

and clotting profile as vital biochemical and haematological changes in snake bite envenomation.

1AIMS AND OBJECTIVES OF STUDY

The study aims to investigate the biochemical parameters associated with snakebite envenomation in the southern part of plateau state , Nigeria.

The specific objectives are as follows;
1. Determine the levels of blood urea and creatinine levels in order to assess increased risk of renal damage in snakebite patients.
2. Assess the levels of serum total proteins and albumin in envenomed patients in order to asses increased risk of liver damage in snake bite patients.
3. Determine the activities of Alkaline phosphate (ALP), Alanine amino Transferase (ALT) and Aspartate Amino-Transferase (AST) in envenomed patients.
4. Determine selected serum electrolytes such as sodium (Na^+), Potassium (K^+), Chloride (Cl^-) and Bicarbonate (HCO_3^-) in envenomed patients
5. To correlate all the selected biochemical parameters in snake bite patients.

II.   MATERIALS AND METHODS
STUDY LOCATION

The site for this study was Jos University Hospital Comprehensive center, Zamko, Langtang North, Plateau State, Nigeria. It has an area of 1,188km^2 and total population of 140,643 at the 2006 census. Langtang North town the headquarter at $9^008'00"N9^047'00"E/9^008N9^047'E$ (PLSG,2017) Residents of the town are mostly business men and women, farmer, hunters, fishermen and civil servants (PLSG, 2017).This hospital is a referral centre in the North-Central for treatment of snakebite envenom patients.

STUDY POPULATION

A total of 243 subjects were used for the study. They were equally grouped into pre-treatment, post treatment an control groups having 81 subjects each.

These patients are those residing in Langtang North and those been referred from other local governments and states within the North-central geographical zone of Nigeria.

INCLUSION CRITERIA

Apparently healthy non-envenom patients from Jos University Teaching Hospital (JUTH) Centre, Zamko and environs were included in this study as control subjects. Age matched envenominated patients were used as test subjects.

EXCLUSION CRITERIA

Patients who had no snakebite record and patients that were bitten by other animals and insects were excluded from this study. Also, patients who were treated from trado-medical houses were excluded from the study.

INFORMED CONSENT

Informed consent was obtained from subjects before sample collection by; self-administered written consent form and questionnaire.

SAMPLE SIZE

A total number of 81 subjects were smapled for each of the control, pre-treatment and post treatment groups.

SAMPLE COLLECTION AND PREPARATION

Five (5)ml of venom blood was aseptically taken from the ante cubital fossa using new disposable pyrogen free needles and syringes with minimum stasis for each pre, post envenomated patients and non envenomated control.

Each blood sample was dispended into a plain sample container (5ml). The blood was kept for 30 minutes to 60minutes in the dark to clot. The samples where then centrifuged at 300rpm for 5 minutes. The sera was separated into clean, plain sample containers in the medical laboratory of the JUTH comprehensive centre, Zamko. The sera were later transported in ice pack cold box to Plateau specialist Hospital, Jos and stored at -20^0C till required analyses for biochemical parameter; electrolytes, Total protein, Albumin, bilirubin, alkaline phosphotase, transaminase, calcium ion, urea and creatinine

LABORATORY BIOCHEMICAL ANALYSIS
CREATININE
The assayed creatinine were done by modified Jaftes method in 1886.
Principle:
Creatinine react with picric acid in an alkaline medium to formed pircrates which is a yellow color
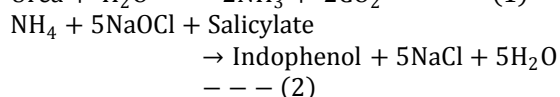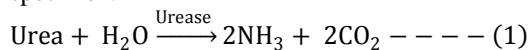
read at 5/onmcolorimetrically.
Method:
    100µL of test and standard was added to the 2ml of working reagent consisting 1ml of alkaline solution and 1ml of picnic acid, mix and incubated at room temperature for 10 minutes and read at 510nm. The creatinine value was calculated using the read absorbance [4].

ESTIMATION OF BLOOD UREA
    The procedure was done according to Berthelot et al with modifications introduced by Fawcett and Scott (Kiranet al., 2004). This method uses urease to hydrolyse urea to produce ammonia and carbondioxide. The ammonia generated reacts with alkaline hypochloride and sodium salicylate in the presence of sodium nitroprusside to form a colored chromaphore. The intensity of the color produced is proportional to the amount of urea in the specimen.

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + 2CO_2 ----(1)$$
$$NH_4 + 5NaOCl + Salicylate$$
$$\rightarrow Indophenol + 5NaCl + 5H_2O$$
$$---(2)$$

SERUM SODIUM AND POTASSIUM (BY FLAME PHOTOMETRY)
    In the flame photometer the solution to be tested is passed carefully under controlled conditions as a very fine spray in the air supply to the burner.
    In the flame, the solution evaporates and the salt dissociated to give neutral atoms. Some of these, though only a very small proportion, move into a high energy state,when these electrons of the atom fall back to their original orbit, they release energy in the form of light, which is used in flame photometry. The dilution for sodium is 1 in 100, for potassium it is usually lower, often 1 in 50, but it is possible by varying the sensitivity to use the same dilution for both salts. Sodium and potassium can interfere with each other. Therefore in standard solutions, both the elements are added [9].
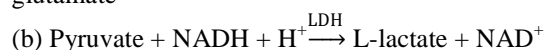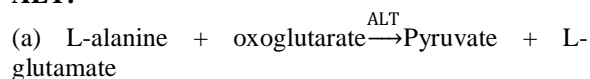
ESTIMATION OF ALANINE AMINOTRANSFERASE (ALT) AND ASPARTATE AMININOTRANSFERASE (AST).
Principle: The estimation of ALT follows the recommendation of the international Federation of Clinical Chemistry (IFCC) and optimized for performance and stability.
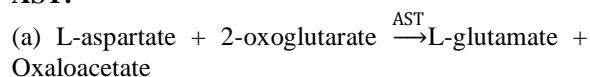   ALT catalyzes the transfer of amino group between L-alanine and L-glutamate or 2-oxoglutarate to form pyruvate. The pyruvate formed, is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD^+. The rate of NADH oxidation is directly proportion to ALT catalytic activity which is measured colorimetrically. It is determined by measuring decrease in absorbance at 340/378nm. Pyridoxalphosphate serves reaction a coenzyme in the amino transfer reaction and ensures maximal activation of enzyme and serves as a preservative.

ALT:
(a) L-alanine + oxoglutarate $\xrightarrow{ALT}$ Pyruvate + L-glutamate
(b) Pyruvate + NADH + H^+ $\xrightarrow{LDH}$ L-lactate + NAD^+

AST:
(a) L-aspartate + 2-oxoglutarate $\xrightarrow{AST}$ L-glutamate + Oxaloacetate
(b) Oxaloacatate + NADH + H^+ $\xrightarrow{MDH}$ Malate + NAD^+
The reaction in ALT/AST is a reversible one and so it is difficult to measure either the reaction substrates or the products, the initial step is coupled with the second step which utilizes NADH as a coenzyme. The oxidation of NADH is followed by measuring the decrease in absorbance at 340nm.

Procedure:
    The tubes were mixed by gentle agitation automatically by cobas CIII chemistry analyzer and samples were incubated at 37^0C for 5 minutes within the analyzer's closed system and decrease in absorbance were taken at 340nm after deionized water was used as a zero calibrator and the analyzer automatically calculates the enzymes activity of each sample and the results recorded stored and printed out.

ESTIMATION OF SERUM ALBUMIN
**Bromocresol Green (BCG) Method**
Principle: Under acidic conditions (pH 4.2) serum albumin binds specifically with BCG to form a green coloured complex. Albumin becomes ionized at an acid pH and thus binds to the anionic dye BCG and absorbance is read at 640nm.

ESTIMATION OF SERUM TOTAL PROTEIN (COLORIMETRIC ASSAY)
Principle: Divalent copper reacts in alkaline solution

with protein peptide bonds to form a characteristic purple-colored biuret complex.
Sodium potassium tartate prevents the precipitation of copper hydroxide and potassium iodide prevents auto-reduction of copper.

Protein + CU^{2+} alkaline solution $\xrightarrow{\text{sodium pottasium tartate /KI}}$ CU − protein colored complex
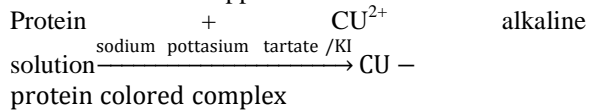
The color intensity formed is directly proportional to the protein concentration which can be determined colorimetrically of 552nm.

Procedure:

The tubes were mixed by gently agitation automatically by cobas CIII chemistry analyzer and samples were incubated at room temperature for 10 minutes within the analyzers closed system and increase in absorbance were taken at 552nm after deionized water was used as a zero calibrator and the analyzer automatically calculates the total protein of each sample and the results recorded, stored and printed out.

DETERMINATION OF SERUM CHLORIDE (MERCUREIC NITRATE ($HgNO_3$), TITRIMETRIC METHOD OF SCHALESS AND SCHALES, 1971))
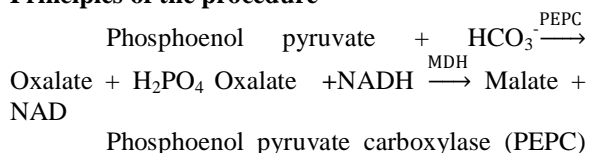**Principle:** When $HgNo_3$ solution is added to a solution containing Cl, unionized but soluble HCl is formed. At the end-point, the first excess Hg+ combine with the indicator dipynelcarbazone to give a violet blue colored complex.
Procedure: To 1.8ml deionized H_2O. Add 0.20ml serum. Then add 3 drops of dipynelcarbazone indicator. Mix well and then titrate from 2ndpipette (graduated on 0.01ml) with $HgNO_3$.
When titration is done directly on plasma, the color at first is salmon red, changing to deep violet when the titration is begun, then becoming pale violet then denotes that end point. Carry out the titration on 2.00ml of the standard chloride solution.

Determination of Serum Bicarbonate (Using Enzymatic Method As Modified By forester et al., 1972)
Principles of the procedure

Phosphoenol pyruvate + $HCO_3^- \xrightarrow{\text{PEPC}}$ Oxalate + H_2PO_4 Oxalate +NADH $\xrightarrow{\text{MDH}}$ Malate + NAD

Phosphoenol pyruvate carboxylase (PEPC)

catalyses the reaction between phosphonel pyruvate and carbon dioxide (bicarbonate) to form oxalacetate and phosphate ion. Oxalacetate is reduced to malate with simultaneous oxidation of an equimolar amount of reduced nicotinamide adenine dinucleotide (NADH) to NAD; the reaction is catalysed by malate dehydrogenase (MDH). This results in a decrease in absorbance at 340nm that is directly proportional to CO_2 concentration in the sample.
Procedure:
Prepare CO_2 reagent according to reagent preparation. Label tubes "blank", "standard", "controls", "patients". Etc. pipette 1.0ml carbon dioxide reagent into each tube. Incubate all tubes for 3 minutes at 37^0 C. Pipette 5µl (0.001ml) of water standard and sample to the cuvette labelled "Blank", "standard" and "patients" respectively. Mix gently by inversion and incubate for 5minutes. Read and record absorbance (Abs) of all cuvettes at 340 nm.
Calculations:
Determine CO_2 content of sample as follows;
CO_2 content of sample (mmol/L) = $\dfrac{\text{Abs. blank} - \text{Abs. sample}}{\text{Abs. blank} - \text{Abs. standard}}$ x concentration of standard.

III. STATISTICAL ANALYSIS

Statistical Package for Social Sciences (SPSS) version 25.0 was used to analyze the results obtained from the research. Means, Standard deviations and standard errors of the various variables were computed. Analysis of Variance (ANOVA) was then used to investigate whether there was a significant statistical difference between the means of the groups in this research. Thereafter, Fisher's Least Significant Difference (F-LSD) was used to determine the means of what group of variables exhibited significant difference in this research. In addition, pearsonscorrelationwas used to test the relationship between all the biochemical parameters.

IV. RESULT

Table 4.1 showed the result analysed for urea, creatinine and electrolytes profile of snake bite patients and controls. The results show that there was significant difference in creatinine ($P < 0.05$). There is no significant differences in other parameters ($P > 0.05$).
Table 4.2; showed the results of some liver function profile of snake bite patients and controls only ALT and AST shows significant difference ($P<0,05$). No significant difference in other parameters ($P >0.05$).

Table 4.3:showed thecorrelation between parameters among control participants. There was no significant correlation among the parameters
Table 4:4; showed the correlation between parameters among participant beforesnake bite treatment significant

correlation between Na^+ and CL~' ALT and CL.
Table 4.5; showed the correlation between parameters among participants after snake bite treatment Significant correlation only exist in urea and AST, urea and ALP, 1C and ALB.

Table 4.1: Urea, Creatinine and Electrolytes Profile of snake bite patients and controls

	Control (n=27)	Before (n=27)	After (n=27)	F- value	p-valuc
Urea (Mmol/L)	3.56±0.10	3.60±0.09	3.69±0.11	2.810	0.662
Creatinine (Mmol/L)	93.70±4.85[a]	85.41±2.25[b]	82.15±2.74[b]	5.712	0.048*
Na^+(Mmol/L)	138.00±0.49	134.63±3.67	138.33±0.58	0.899	0.411
K^+ (Mmol/L)	3.84±0.07	3.99±0.06	4.04±0.09	2.205	0.117
HCO_3^-	24.41±0.60	25.67±0.72	26.37±0.59	1.972	0.146
Cl^-(Mmol/L)	102.41±0.65	102.04±0.65	102.26±0.67	0.082	0.921

Note:
The Critical F= 3.112
Different alphabets in superscripts in rows, showed the significant difference between the meanof the treatments and control after Fisher's Least Significant Difference (F-LSD) Post-Hoctest
Key: Na^+- Sodium ion , K^+= Potassium ion, HCO_3^- = icarbonate ion, Cl- = Chloride ion * = significant value

The result from Table 4.1 showed that there is no significant difference ($p>0.05$) in all theparameters examined except creatinine (mmol/L) which showed significant difference($p<0.05$). The Post hoc test revealed that the significant difference is between control, beforeand after snake bite. The control subjects have a higher mean Creatinine concentration(93.70±4.85) than those bitten by the snakes either before treatment (85.4l±2.25) or aftertreatment (82,15±2.74).

Table 4.2; Some liver function profile of snake bite patients and controls

	Control (n=27)	Before (n=27)	After (n=27)	F- value	p-value
TP (g/I)	77.15±1.83	78.70±1.46	77,85±1.83	0.206	0.814
ALB (g/1)	33.00±0,79	33.89±0.79	35.26±1.01	1.724	0.185
ALT (U/L)	8.93±0.71[b]	10.41±0.70[b]	12.93il.42[11]	4.058	0.021*
AST (u/L)	I0.00±0.77[b]	10.85±0.82[b]	18.44±1.66[a]	10.372	0.001*
ALP(1U/L	76.85±2.27	76.26±1.94	74.07±2.04	0.491	0.614

Note:
The Critical F~ 3.112
Different alphabets in superscripts in rows, showed the significant difference between the meanof the treatments and control after Fisher's Least Significant Difference (F-LSD) Post-Hoctest
Key: TP = Total protein, ALB = Albumin, ALT = alanine amino transferase, AST= Aspartate Ammo transferase, ALP = Alkaline phosphatase.

The result from Table 4.2 showed that there is no significant difference ($p>0.05$) in all the liver function profile examined except ALT and AST which showed significant difference ($p<0.05$). Post hoc test revealed that ALT and AST were significantly increased after the post-treatment of the snake bite envenom. This is an indication that the treatment triggers an increased levelsof ALT and AST above control level.

Table 4.3: Correlation between parameters among control participants

	Urea	Creatinine	TP	Na	K	ALB	ALT	AST	ALT	Cl-	HCO$_3$
Urea	1										
Creatinine	0.163	1									
TP	-0.169	0.162	1								
Na	-0.006	-0.066	-0.290	1							
K	-0.340	-0.237	-0.245	0.226	1						
ALB	-0.027	-0.024	-0.273	0.015	0.328	1					
ALT	0.209	-0.032	0.378	-0.224	-0.117	-0.109	1				
AST	0.103	-0.218	0.250	-0.120	-0.144	0.002	0.272	1			
ALT	0.125	0.003	-0.085	-0.069	0.229	0.083	0.161	-0.134	1		
Cl-	-0.225	-0.078	-0.127	-0.058	0.181	-0.086	-0.307	0.280	0.000	1	
HCO$_3$	0.070	0.156	0.231	-0.160	0.234	-0.015	0.157	0.090	0.053	-0.196	1

Table 4.4: Correlation between parameters among participants after Snake bite

	Urea	Creatinine	TP	Na	K	ALB	ALT	AST	ALP	Cl$^-$	HCO^3
Urea	1										
Creatinine	0.156	1									
Na	0.086	0.023	1								
K	0.055	0.150	-0.106	1							
ALB	-0.289	-0.068	0.230	0.204	1						
ALT	0.095	-0.159	0.233	-0.186	0.137	1					
AST	-0.032	-0.013	0.067	-0.062	0.056	-0.246	1				
ALP	-0.112	0.232	0.281	0.075	0.143	0.003	-0.157	1			
Cl$^-$	0.232	0.006	0.105	0.143	-0.137	0.318	0.102	-0.084	1		
HCO^3	0.153	0.287	0.182	-0.386*	0.196	0.280	-0.436	0.197	0.048	1	
Creatinine	0.103	0.159	0.095	0.128	0.040	0.073	0.153	-0.196	0.186	0.230	1

Note:
The significant values are denoted by asterisks.
*=P<0.05 (significant)
**=P<0.01 (Highly significant)

Table 4.5: Correlation between parameters among participants after Snake bite

	Urea	Creatinine	TP	Na	K	ALB	ALT	AST	ALT	Cl$^-$	HCO^3
Urea	1										
Creatinine	-0.199	1									
TP	-0.261	0.001	1								
Na	0.222	-0.002	0.134	1							
K	0.215	-0.103	-0.080	0.058	1						
ALB	0.237	-0.185	-	-	0.415*	1					

			0.076	0.258							
ALT	0.073	-0.009	-0.130	-0.030	0.317	0.257	1				
AST	0.541**	-0.336	-0.208	-0.034	0.012	0.009	0.066	1			
ALT	0.382*	-0.048	-0.069	-0.046	0.132	0.230	0.165	-0.087	1		
Cl⁻	-0.067	-0.215	-0.324	-0.119	-0.177	0.104	-0.152	-0.203	0.187	1	
HCO^3	-0.046	-0.287	0.104	-0.006	0.247	-0.068	0.133	0.214	-0.008	-0.092	1

Note:
The significant values are denoted by asterisks.
*=P<0.05 (significant)
**=P<0.01 (Highly significant)

V.   DISCUSSION, CONCLUSION AND RECOMMENDATION

DISCUSSION

This study was designed to evaluate some blood biochemical parameters in pre (before) and post (after) snake bite envenom in subjects and control of apparently healthy subjects within southern part of Plateaus state, Nigeria. Jos University Teaching Hospital (JUTH) comprehensive centre, zamko branch in Lantang Northwas used as collection centre since it served as referral centre for snake treatment.

From the study conducted, the mean urea values were within normal range across the control subjects, pretreated subjects and post -treated subjects. The P-value indicated that there was no significant difference between the snake bite subjects as there was no raised in urea (P> 0,05) compared to the control. These observations are similar to the previous work that indicated that urea was lowered as a result of venom not sufficient to cause kidney damage and such patients do not require admission [17].Also there are no significant mean Urea is differences between pre-treated and post-treated subjects (P> 0.05). This finding is in accord with the earlier findings which indicated that snake bite-induced acute kidney infection (AKI) with rapid raise in blood urea [18].

From the study of Sodium ion the mean values showed no difference across the control subjects, pre-treated subjects and post treated subjects. Also, there was no raised in Na+ of the snakebite subjects (P>0.05) compared to the control subjects. This is similar with the previous work that indicated that results of Na$^+$ estimation showed no significance increase and low level of Na$^+$ was observed Mowing Kraft snakebites [7].

Also the pre-treated and the post treated subjects exhibited no significantdifferences (P>0.05). This finding tally with the earlier work that stated results of Na$^+$ estimation showed no significant increase in cases of viperbite compared to the control and if there is any increase in Na$^+$ may be due to the secondary effect of renal failure [8].From the study of the serum potassium ion (K$^+$) the mean values were within normal range across the subjects; control, pre-treatment, and post-treatment and the result showed no statistical increased in K$^+$ (P >0.05); compared with the control subjects. This study is in tandem with the previous studies conducted in Sri Lanka by [7].where hypokaiemia was observed following krait bites and studies conducted by Kumar et al shown that there was not significant increase serum potassium level in both viper and cobra bites cases [8].The pretreated and post -treated subjects exhibited no significant differences (P = 0,117; P >0.05). This findings is in accord with the earlier work indicated that in the absence of renal impairment hypokaiemia could be due to increased urinary excretion or intracellular shifting of potassium [6].

From the study of Bicarbonate (HCO$_3$) the means values were within normal range between control and pre-treated subjects. There was no statistical mean differences between the control and post -treatment subjects(p>0.05). This does not agree with the previous work of [16]that established acute renal failure, administration of sodium bicarbonate can be dangerous and should avoided due to fluid overload and hyperosmololity.

The study of chloride ions (Cl-) the mean values were all normal across thecontrol, pre-treated and post-treated subject and there was no significant rise in CL~of the snakebite subjects (P> 0.05)

compared with the control. This agrees with theprevious work that indicated that patients with AKI had a significantly lower level

of serum chloride [9].

In table 4.2, the total protein (TP) mean values were within the normal range across all the control, pre-treated, post-treated subjects and the result below from the P-value was insignificant There was no raised in total protein (TP) of the snake bite subjects (P>0.05) compared to the control subjects. This observation is similar to previous work their indicated that permeability, together with renal damage would further aggravate the accompanying hypoproteinemia[15].Also, the pre-treated subjects and the post-treated subjects exhibited no significant differences asProtein was not increased (P= 0.814, P> 0.05). This findings is in accord with the earlier work that indicated that hypoproteinemia can be associated with severity of snake bite [5].

Albumin (ALB) mean values were within the normal range across all the control, pretreated post-treated subjects and the result below from the P-value was insignificant as there were no raise in albumin of the snake bite subjects (P>0.05)compared to the control subjects. This observation is similar to the previous work that indicated that renal damage would further decreased the level of albumin in the blood [15].Also, the pretreated subjects and post-treated subjectsexhibited no significant differences as albumin as was not increased, P > 0,05). Thisfindings is in accord with the earlier work that indicated the hypoalburninaemia isbeen associated with the severity of the snake bite [5].from the study alanine aminotransferase mean values were within normal range between control subjects an pretreated subjects and between pre-treated and post-treated subjects. There were mean value differences between control subjects and post-treated subjectseven though not statistically significant. The result of P-value indicated a significant differences as there was raised in ALT of the snake bite subjects (P<0,05) comparedwith the control subjects. The observation is related to the previous work thatindicated that clinical observations that experimental mice could develop hepaticdisease after snake bite envenoming thereby increased level of ALT [9].Also, there exhibited a significant differences between the pre-treated subjects and post-treated subjects (P<0.05). This findings agrees with the earlier work that indicated that envenomation following viper bite can result to hepatocellular injury thereby increased ALT level and this may develop slowly over

several days [16].From the study aspartate amino transferase (AST) mean values were within the normal range between control and pre-treated subjects and there was a significant mean differences (P<0.05) between the control subjects and the post-treated subjects. Also, significant mean differences exist between pre-treated subjects and post-treated subjects and the result from P-value indicated a significant difference as there was raised in AST of the snake bite post-treated subjects (P<0.05) compared to the control subjects. This observation is similar to the previous work that indicated that clinical observations have suggested that dogs and experimental mice could develop hepatic disease after snake envenoming thereby increased level of AST [10].Also, there exhibited a significant difference between the pre-treated subjects and post-treated subjects (P <0.05). This findings is related with the earlier work that indicated that envenomation following viper bite can result in hepatocellular injury thereby increased AST level and these may develop slowly over several days [9].

In the study, alkaline phosphatase mean values across the control, pre-treated and post-treated subject! significant. The result from the P-value below was insignificant as there was no raised in alkaline phosphatase of the snake bite subjects (P>0.05) compared to the control subjects, this observation is similar to the previous work that indicated that in the absence of hepatic hysfunction serum ALP is always low, were there is hepatic dysfunction serum ALP is always low, were there is hepatic dysfunction serum ALP increased [12].The pre-treated and post treated subjects exhibited no significant differences as ALP was not raised (P > 0.05). This findings agreeswith the earlier work that indicated that envenomation following viper bite can result to hepatocellular injury thereby increased level of the enzymes otherwise will remained normal [7].

Table 4.4 showed the correlation matrix of all the variables of participants before snake bite treatment. The result showed that two pairs of variables were significant (P<0.05). These include Na+ vs Cl-; and ALT vs Cl" with r= -0,386 and -0.436 respectively. These indicated that there were negative correlations between the pair of variables identified. An increased Na+ will lead to a decrease in Cl" and vice-versa. Likewise an increase in ALT will lead to decrease in Cl" and vice versa. Meanwhile, all other variables have no significant correlation (P>0.05). This relation result partially agreed with the previous work that indicated that results of Na+ showed no significant increase in cases of viper bite compared to the control and if there is any increase in Na+ may be due to the

secondary effect of renal failure (Kumar et al, 2011).

In addition, this result is similar to the previous work that indicated that clinical experimental mice could develop heptic disease after snake envenoming thereby increased level of ALT [7].The decreased in Cl* of the pre- treated snake bite subjects as correlated with ALT agrees with the previous work that indicated that patients with acute renal injury had a significantly lower level of serum Cl- [11].

Table 4.5 showed the correlation matrix of all the variables of participants after snake bite treatment. The result showed that three pairs of variables were" significant (P<0.05). These include ALB vs K+; AST vs Urea and ALP vs Urea with r= 0.415,0.541 and 0382 respectively. These indicated that there were positive correlation between the pair of variables identified. An increased ALB will lead to an increase in K+ and vice-versa. This findings is in accord with the-previous work that indicated that hypoalbuminaemia is been associated with the- severity of the snake bite [12].Also the findings tally with the earlier work indicated in the absence of renal Impairment hypokalemia could be due to increased nary excretion or intracellular shifting of K+[12].Likewise an increase in AST will lead to increase in Urea and vice versa. In addition, an increased F*

ALP will lead to an increase in Urea and vice-versa. Meanwhile, all other variables have no significant correlation (P>0.05).

It could be. deduced from the correlation tables in 4.4.and 4,5 that treatment of snake venom alters the biochemical concentration such that an indirect relationship becomes a direct relationship.

CONCLUSION

Many cases of research about snakes bite such as in the vipers which are responsible for the accident are not frequently reported. However, this research work has provided information to clarity the various clinical laboratory methods used in the diagnosis of some biochemical parameters in snake bite cases. Some of these parameters were able to factor out those organs that are damaged by snake venom. Most of the organ have been affected by the vipers snake venom include; Kidney, liver and heart,

Based on the result of this research, we concluded that the biochemical parameters that were statistically significant in vipers snake bite envenomed patientsinclude creatinine, aianinetransamines (ALT) and aspartate transaminases (AST). While there are significant indirect relationship between

pair parameters such as Na+ vs Cl-;and ALT vs Cl" in participants before snake bite treatment, there are positive/direct significant relationship between pair of variables such as ALB vs K+; AST vs Urea and ALP vs Urea in participants after snake bite treatment. These parameters are very important to this study and they are significant in biochemical diagnoses of snake bite envenom patients in southern plateau, Nigeria,

RECOMMENDATIONS

Since snake bite envenoming constitutes an important public health problem in parts of southern plateau, Nigeria, there should be important progress toward generating a better understanding of snake venoms. Also, snake bite envenoming, clinical biochemical diagnoses of the snake venom and their treatment can be made through; efforts in research, antivenom availability and accessibility.

Since the viper snakes in southern plateau can camouflage, farmers and hunters should always use their rubber boots while clearing the farms and should avoid deeping their hands inside holes as this serves as hiding places. The people should clear tall vegetation close to their settlements. Rocky areas should also be properly cleaned to avoid snakes form hiding.

Finally, the government from time to time can fumigate those areas that harbour' s the snakes and support by paying bills of those patients with snake bites because most of them are poor farmers and hunters.

CONTRIBUTION TO KNOWLEDGE
This study has contributed to knowledge in the following ways:

It was discovered that the Creatinine was significantly higher in snakebite envenomed subjects and this could be as a result of kidney damage. Secondly, the study revealed that enzymes such as alanine aminotransferase (ALT) aspartate aminotransferase (AST) always increased in snake bite subjects; this could be as a result of liver damage caused by the snake venom.

REFERENCE
[1]. Aguiyi, J. C. Igweh, A. C., Egesie, U. G., Leocini, R., (1999). Studies on possible protection against snake venom using Mucunapruriens.protein immunization Fitoterapia 70:21-26.
[2]. Amaral, C.F., Campolina, D., Dias, M.B, Bueno, C.M. and Rezende, N.A. (1998). "Tourniquet ineffectiveness to reduce the

severity of envenoming after Crotalusdurissus snake bite in Belo Horizonte, Minas Gerais, Brazil". Journal of toxinology.36 (5): 805–808.

[3]. Bon, C., Arocas, V., Braud, S., Francischcti, I. and Leduc, M. (2000) Snake venom in thombosis and haemostatis. XII the word congress of the International Society of Toxinologie, Paris, September: 18-22.

[4]. Chippaux, J.P. (2008) Snake-bites: appraisal of the global situation, World Health Organisation. 76: 515–524.

[5]. Feish, T.W., Gold, B.S. Richard, C. Dart; Robert A. Barish (1 April 2002). "Bites of venomous snakes".The New England Journal of Medicine.347 (5): 347–56

[6]. Harish, R.T., Paine, M.J., Desmond, H.P, Theakston, RDG, Crampton JM (2020) Purification, cloning and molecular characterization of a high molecular weight hemorrhagic metalloprotease, jararhagin, from Bothropsjararaca venom. Insights into the disintegrin gene family.Journal of Biological Chemistry 267: 22869-22876.

[7]. Kularatne, Y.T., Marsh, N. and Whaler, B. (2002) The effects of snake venoms on the cardiovascular and haemostatic mechanisms. International Journal of Biochemisty, 9: 217-220.

[8]. Kumar, G.Y., Lomonte, B., Gutiérrez, J.M., Borkow, G., Ovadia, M. and Tarkowski, R. (2011) Activity of hemorrhagic metalloproteinase BaH-1 and myotoxin II from Bothropsasper snake venom on capillary endothelial cells in vitro. Journal of Toxinology,32: 505-510.1994.

[9]. Kyi – phyu, F.T., Retzios AD, Markland FS., Jr (2017). A direct-acting fibrinolytic enzyme from the venom of Agkistrodoncontortrixcontortrix: effects on various components of the human blood coagulation and fibrinolysis systems. Thrombosis Research, 52:541–552.

[10]. Laing, G.D. and Moura-da-Silva, A.M. (2005) Jararhagin and its multiple effects on hemostasis. Journal of Toxinology, 45: 987-996.

[11]. Mohapatra, R., Mathers, C.D., Ezzati, M. and Lopez, A.D. (2011) Measuring the Burden of Neglected Tropical Diseases: The Global Burden of Disease Framework. PLoS Neglected Tropical Disease. 14: 2545-2558.

[12]. Nordt, S.P. and Clark, R.F. (2001). Rattlesnakes and other crotalids. In: Ford MD, Delaney KA, Ling LJ, Erickson T, editors. Clinical toxicology. 1st ed. Philadelphia: WB Saunders. pp. 863–872.

[13]. Oshaka, A. (1979) An approach to the physiological mechanism involved in haemorrhagic principles as a useful analytical tool. Animal, plant and microbial toxins 123: 17-28.

[14]. Patrick, J, Morrison, R; Stewart, R; Gore, D (2013). "Venomous bites and stings".Current Problems in Surgery.50 (1): 9–44

[15]. Sakai, A. (2013),.Diagnosis and treatment of snakebite by Mamushi and Yamakagashi.Journal of Pharmacology and Experimental Therapeutics 26:193–199.

[16]. Sakwiwatku, T.D., Qiu Y, Choo YM, Yoon HJ, Jin BR (2002) Molecular cloning and fibrin(ogen)olytic activity of a bumblebee (Bombus hypocrite sapporoensis) venom serine protease. Journal of Asia-Pacific Entomology, 15: 79-82.

[17]. Spano, T.G., Norris, R.L, Dery, R., Johnson, C., Williams, S. and Rose, K. (2013) Regional vs systemic antivenom administration in the treatment of snake venom poisoning in a rabbit model: a pilot study. Wilderness Environmental Medicine, 14: 231-235

[18]. Vikrant, J.T., Nuchpraryoon, I. and Garner, P. (2017). "Interventions for preventing reactions to snake antivenom". The Cochrane Database of Systematic Reviews (2):35-39.

[19]. Warred, R., Sharma SK, Chappuis F, Jha N, Bovier PA, Loutan L. (1984) Impact of snake bites and determinants of fatal outcomes in southeastern Nepal. American Journal of Tropical Medical Hygiene, 6(2): 23-29.

[20]. WHO (2010) Neglected tropical diseases: snakebite. P. 45.

[21]. Williams D, Gutierrez J-M, Harrison RA, Warrell DA, White J. (2009) An antidote for snake bite: The Global Snake Bite Initiative. Lancet,3(1)23-35.