



Research Article

Effect of the *Bitis arietans* snake venom obtained from Kaltungo, Nigeria on Liver Oxidative Stress Status and Histological changes in Wistar Albino Rats

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ABSTRACT

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The venom of the Nigerian *Bitis arietans* (puff adder) was assessed for its ability to trigger venom-induced necrosis as well as oxidative stress in the liver of Wistar albino rats. The snake was captured in Kaltungo Gombe state and the venom was milked and lyophilized accordingly. A single LD₅₀ dose (1.5mg/kg) was administered and the rats were monitored for two weeks and the liver homogenate samples were analyzed for the changes in oxidative stress markers. Histopathology of the liver tissue was conducted to assess the necrotic effect of the venom. The Malondialdehyde (MDA) values were not significantly changed while the superoxide dismutase (SOD) and reduced glutathione (GSH) levels were affected significantly. One week after venom administration, the branching and anastomoses of the hepatocytes in the liver tissue were observed to be not clearly demarcated due to the severity of the necrosis with disintegrated nuclei especially in the wall of the central vein. There was almost total disintegration of the wall of the central vein two weeks after venom administration. The results of this study indicate that the Nigerian puff adder venom can also trigger oxidative stress and promote necrosis in the liver tissue of Wistar albino rats.

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INTRODUCTION

Puff adder (*Bitis arietans*) is a large venomous snake found in many parts of Africa and Arabia. It belongs to the viper family and possessed an extremely toxic venom containing cytotoxic components (Britannica, 2020). This family of snakes is a good swimmer and can climb trees (Mallow *et al.*, 2003). A large female puff adder can produce up to 80 snakes through ovoviviparous means (Mehrten, 1987). The typical size of the adult snake is up to 1.0 m in length. In a single puff adder envenomation, venom yield of about 150 to 350 mg can be injected (Mallow *et al.*, 2003). This can result in severe swelling and cellular damage leading to necrosis (Mallow *et al.*, 2003). Like other venomous snakes, the venom composition of puff adder depends on diet, age as well as region (Tasoulis, 2017; Reeks *et al.*, 2015). In general, their venom is composed of metalloproteinase, serine proteases, other enzymes and some non-enzymatic proteins (Rivel, 2016). The presence of L-amino acid oxidase (LAAO) in the venom can lead to venom-induced oxidative stress (Tan, 2015). The cytotoxins in viper venoms display activity on different cell types, including spleen cells, lymphocytes, erythrocytes, cardiac myocytes, and different tumour cells (Stevens-Truss, *et*

al., 1996). The pathological consequences, however, depending on the cell membrane proteins and phospholipids found on the outer leaflet of the plasma membrane of these cell types (Dufton and Hider 1998). Cytotoxins possess a relatively high affinity for negatively charged lipids (Gasarov *et al.*, 1997).

Some cytotoxins can change lipid-induced oligomerization (Forouhar, *et al.*, 2003). Their interaction with negatively charged lipids can lead to the dimerization of cytotoxins, which can result in oligomerization, a critical step in the formation of membrane pores. Hence, this is the molecular basis for the formation of membrane pores (Harvey, 1990). Once thought to exist only as monomers, cytotoxins are now known to form dimers, which can synergize downstream pathology on targeted cells. A neurotoxic dimer containing a cytotoxin was isolated from *Naja kaouthia* venom (Osipov *et al.*, 2008). This dimer consists of a three-fingered α -neurotoxin bound to cytotoxin via disulfide bonds. The cytolytic activity of the cytotoxin is completely lost upon its association with α -neurotoxin (Osipov *et al.*, 2008).

This study is aimed at further investigating the possible pathophysiological effects *Bitis arietans* venom could cause at a known LD₅₀ dose in Albino rats.

MATERIALS AND METHODS

Experimental Animals

Exactly 40 Wistar albino rats weighing 150 to 200 g were used. The animals were obtained from the large animal facilities, National Veterinary Research Institute Vom, Plateau State, Nigeria. They were fed and watered *ad libitum* throughout the study. An ethical clearance \

(AEC/02/74/19) on the use of animals was obtained from the National Veterinary Research Institute Vom, Plateau State, Nigeria.

Venom Collection and lyophilization

A live male *Bitis arietans* snake (puff adder), aged 4 to 5 years was captured in Kaltungo local government, Gombe State Nigeria and was identified by a herpetologist at the Faculty of Veterinary Medicine, Ahmadu Bello

University Zaria and Gombe State University and a voucher number (VBA: 005) was allocated. The snake was grasped firmly at the back of the head and was made to release its venom into a graduated conical flask which was immediately kept at 4oC in preparation for lyophilization (Allen and Eugene 1941). The venom was then lyophilized using an LGJ-12 freeze dryer at National Research Institute for Chemical Technology Zaria, Kaduna State.

Experimental Grouping and Venom Administration

The 40 albino rats were grouped into 4 (n10): control, group one, group two and group three. All were administered the same LD50 dose of 1.5mg/kg orally or intraperitoneally except for the control. Groups one, two and three were sacrificed 24 hours, 7 days and 14 days respectively. The blood and liver were harvested and stored accordingly. To monitor the effect of the venom LD50.

Estimation of Oxidative Stress Markers

Reduced glutathione (GSH) level was estimated spectrophotometrically using the method described by Betler *et al.* (1963). The liver homogenate was deproteinated using 4% sulfosalicylic acid. After centrifugation at 4,000 xg for 5 minutes, the Ellman reagent was added to the supernatant and the absorbance

was measured at 412 nm. Malondialdehyde (MDA) was estimated as described by Varshney and Kale (1990). The samples in tris-KCl buffer were mixed with 30% TCA and 0.75% TBA and placed in a water bath for 45 minutes at 800C. It was then cooled in ice and centrifuged at 3000 g for 5 minutes and the supernatant was used to estimate MDA levels at 532 nm. Lipid peroxidation expressed as MDA formed/mg protein or gram tissue was computed with a molar extinction coefficient of 1.56 x 10⁵ M⁻¹ Cm⁻¹. Determination of superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972). A 1 in 5 dilutions of the samples was made to which 0.05 M carbonate buffer (pH 10.2) and freshly prepared 0.3 mM adrenaline were added to the mixture which was quickly mixed by inversion. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

Statistical Analysis.

The values for the oxidative stress parameters were expressed as mean ± SEM and analysed statistically between-group using one way ANOVA with the aid of SPSS version 16 software and a P value of 0.05 was significant.

RESULTS

Table 1: Venom effects on Oxidative Stress Markers in Wistar Albino Rats

Markers	Control	Day one	Week one	Week two
MDA	1.817x10 ⁻⁵ ±2x10 ^{-6a}	1.808x10 ⁻⁵ ±1x10 ^{-6a}	1.831x10 ⁻⁵ ±1x10 ^{-6 a}	1.586x10 ⁻⁵ ±0.0 ^a
GSH	3.20±0.25 ^b	3.75±0.79 ^c	3.60±0.44 ^c	18.25±3.14 ^d
SOD	42.26±3.13 ^e	55.037±4.12 ^f	56.29±6.88 ^f	36.99±7.83 ^g

Values with different superscript are significantly different from each other at P < 0.05 between groups for each oxidative stress marker
 MDA: malondialdehyde; GSH: reduced glutathione; SOD: superoxide dismutase; All values were expressions of two independent experiments.

Effect of LD 50 dose administration *Bitis arietans* snake venom on liver Histopathology

At day one.....

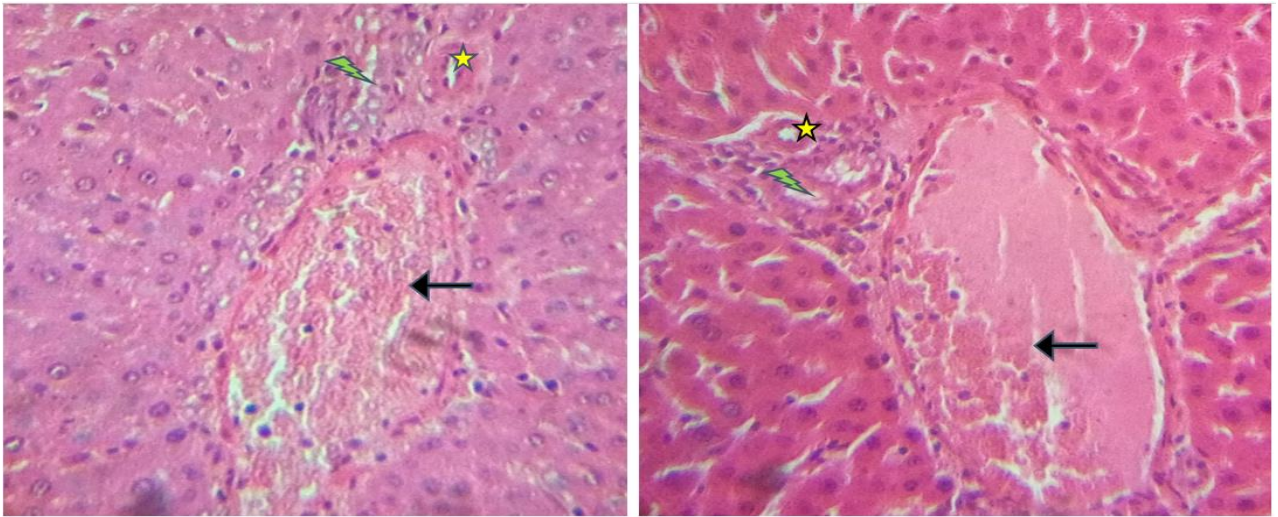


Figure 1: Rat liver showing congestion of the portal vein (Black Arrow), the hepatic artery (yellow stars) and the bile duct (green thunder bolt). The necrosis of the hepatocytes is mild . The portal vein, the hepatic artery and the bile duct form the Portal triad. Magnification x100

At week one.....

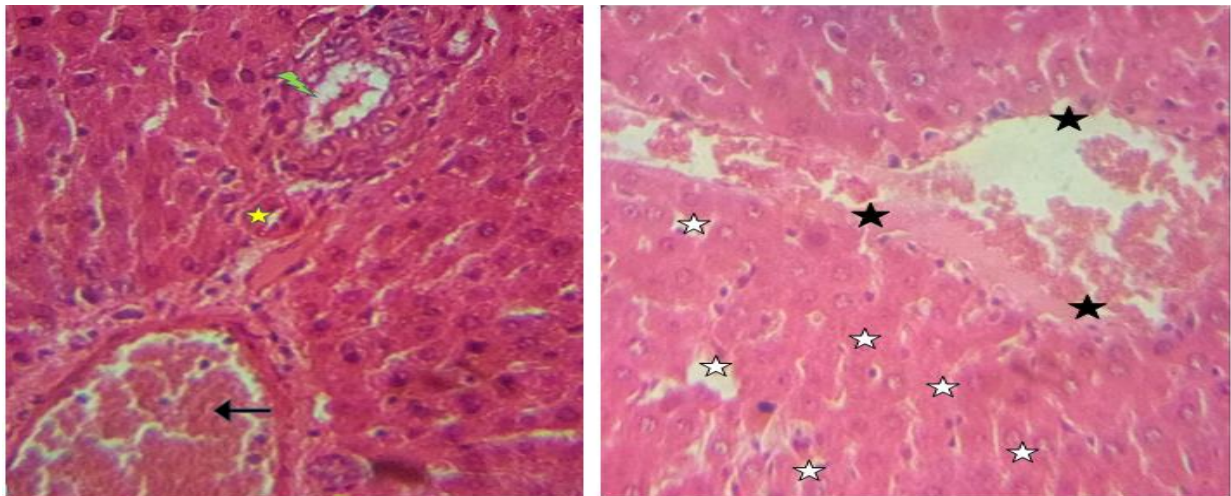


Figure 2: The Portal vein is congested (black arrow). The branching and anastomoses of the hepatocytes are not clearly demarcated due to the severity of the necrosis with disintegrated nuclei (white stars). There is sever necrosis of the wall of the central vein (Black stars). The portal vein, the hepatic artery (Yellow star) and the bile duct (Green thunder bolt) form the Portal triad. Magnification x100

At week two.....

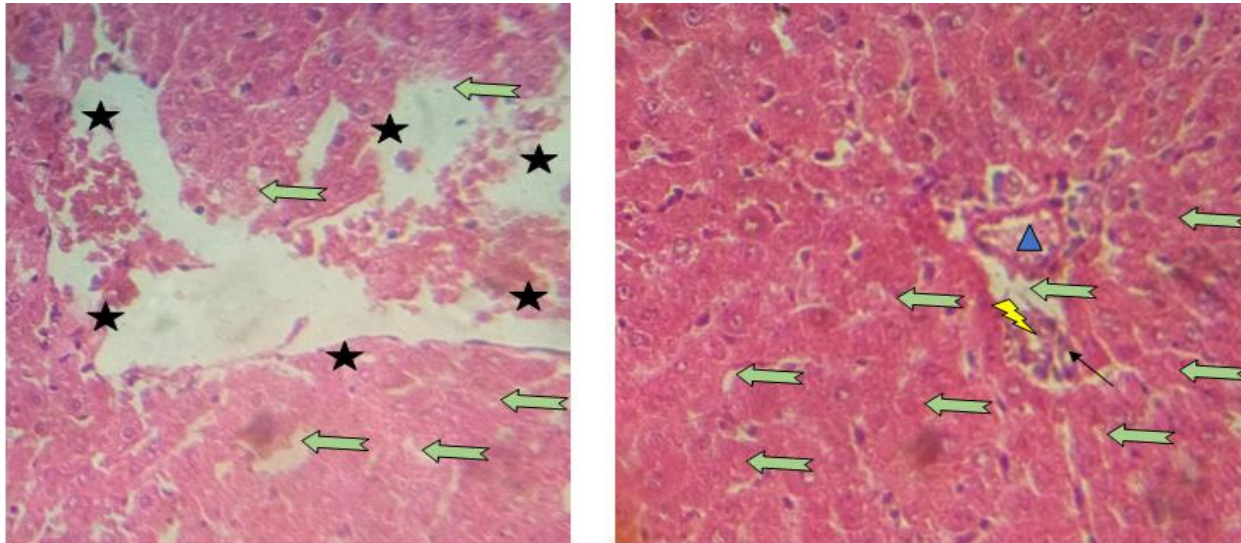


Figure 3: There is severe necrosis of the hepatocytes. The branching and anastomoses of the hepatocytes are not clearly demarcated due to the severity of the necrosis with disintegrated nuclei (Green arrows). There is almost total disintegration of the wall of the central vein (Black stars). The portal vein (Blue triangle), the hepatic artery (Yellow thunder bolt) and the bile duct (Black arrow), form the Portal triad. Magnification x100

DISCUSSION

Levels of oxidative stress biomarkers of lipid peroxidation MDA, GSH and (SOD) were assessed in liver homogenate. Lipid peroxidation indicates oxidative damage caused by ROS and suggests cytotoxic effects of the venom. Changes in the level of lipid peroxidation suggested an imbalance reaction between oxidants and antioxidants in the biological system (Frezzetti and Silveira, 2011). The lack of significant changes in MDA level observed (Figure 1) between the control and the other groups could be due to the route of envenomation preventing the bioavailability of some venom components (Sanhajariya, *et al.*, 2018). The marker GSH is a reduced form of tripeptide glutathione, and it is a potent intracellular antioxidant defence system that

acts by directly acting on reactive oxygen/nitrogen species (Dickinson and Forman, 2002). The significantly increased GSH level observed (Figure 2) could be suggestive of the presence of potentially toxic venom components. According to Thom *et al.* (1997), a rise in GSH level could be due to an increase in RBC destruction (hemolysis) which can be triggered due to hemotoxic venom components. The changes in glutathione levels serve as an indicator of oxidative stress (Bauche, *et al.*, 1994) and are a signal for expressing and regulating detoxification (Kirlin, *et al.*, 1999). However, the GSH level returning to the normal level after one and two weeks suggests that the rats were capable of recovering from the toxic effects of the venom at the dose used for this study. The increased

level of superoxide dismutase (SOD) (Figure 3) observed could be due to the presence of free radicals such as hydrogen peroxide (H₂O₂) and molecular oxygen (O₂) which can be a result of damaging effects of the venom phospholipases and L amino acid oxidase in the venom that catalyses the oxidative deamination of L-amino acid to alpha-keto acid stereo-specifically to liberate ammonia and hydrogen peroxide (Du and Clemetison, 2020; Singh *et al.*, 2017; Sies, 2017). The decreased level observed two weeks after venom injection could be due to the decreased effect of the venom proteins, especially L amino acid oxidase.

The mild necrosis observed 24 hours after venom administration could be due to the limited bioavailability of the venom. This is a usual venom effect when administered subcutaneously. However, the increase in necrosis observed beyond one week after venom administration could be due to delayed bioavailability of the venom components released at gradual phases (Sanhajariya *et al.*,

2018). The primary necrotic factors that could be responsible are proteins and peptides. These include phospholipases and other proteins (Sani *et al.*, 2019). Among the families of phospholipases are phospholipase A2. This group of enzymes are capable of hydrolyzing the membrane lipid which triggers a cascade of inflammatory reactions involving pro-inflammatory lipid mediators (Jia *et al.*, 2020).

CONCLUSION

Snake envenomation is one of the leading causes of death due to multiple organ failure among the rural population. Because snakes are region-specific, the establishment of revealing the pathological effect of indigenous puff adder is paramount. This study indicates the possible time-dependent liver necrosis caused by puff adder regardless of changes in the oxidative stress markers over time and hence could guide the physician to carry out a proper treatment approach.

REFERENCE

- Betler, E., Dron, O. and Kelly, B.M. (1963). Improved method for the determination of blood glutathione. *J Lab Clin Med.* 61:882-8.
- Britannica. The Editors Encyclopaedia. "Gaboon viper". *Encyclopedia Britannica*, 6 Jun, 2020. <https://www.britannica.com/animal/gaboon-viper>. Accessed 13 December 2021
- Chethankumar, M. and Srinivas, L., (2008). Gangliosides as potential inhibitors of *Naja naja* venom PLA2 (NV-PLA2) induced human erythrocyte membrane damage. *Afr J Biochem.* 2(1):8–14.
- Dickinson, D.A. and Forman, H.J. (2002) Cellular glutathione and thiols metabolism. *Biochem. Pharmacol*, 64, 1019–1026.
- Du, X.Y. and Clemetson, K.J., 2002. Snake venom L-amino acid oxidases. *Toxicon*, 40,659-665. *Advances in Microbiology*, vol.4 No.16.
- Dufton, M.J. and Hider, R.C. (1988). Structure and pharmacology of elapid cytotoxins. *Pharmacology and Therapeutics.* 36:1–40.
- Dufton, M.J. and Hider, R.C. (1988). Structure and pharmacology of elapid cytotoxins. *Pharmacology and Therapeutics.* 36:1–40.

- Forouhar, F., Huang, W.N., Liu, J.H., Chien, K.Y., Wu, W.G. and Hsiao, C.D. (2003). Structural basis of membrane-induced cardiotoxin A3 oligomerization. *Journal of Biological Chemistry*. 278:21980–21988.
- Frezzatti, R. and Silveira, P.F. (2011). Allopurinol reduces the lethality associated with acute renal failure induced by *Crotalus durissus terrificus* snake venom: comparison with probenecid. *PLoS. Negl. Trop. Dis.* 5, e1312.
- Gasarov, S.E., Alsarraj, M.A., Gasarov, N.E. and Rael, E.D. (1997). Cobra venom cytotoxin free of phospholipase A2 and its effect on model membranes and T leukemia cells. *Journal Membrane Biology* 155:133–142.
- Harvey, A.L. (1990). Cytolytic toxins. *Handook of Toxicology*. Marcel Dekker Inc; New York, USA. p. 66.
- Jia Jin Hiu, Michelle Khai and Khun Yap. (2020). Cytotoxicity of snake venom enzymatic toxins, phospholipase A2 and l-amino acid oxidase. *Biochem Soc Trans*; 48 (2): 719–731.
- Kirlin, W.G., Cai, J., Thompson, S.A., Diaz, D., Kavanagh, T.J. and Jones, D.P. (1999). Glutathione redox potential in response to differentiation and enzyme inducers. *Free Radic Biol Med*; 27: 1208–1218.
- Mallow, D., Ludwig, D. and Nilson, G. (2003). *True Vipers: Natural History and Toxinology of Old World Vipers*. Krieger Publishing Company, Malabar, Florida. 359 pp. ISBN 0-89464-877-2.
- Mehrtens, J.M. (1987). *Living Snakes of the World in Color*. New York: Sterling Publishers. Pp 480.
- Misra, H.P. and Fridovich, I. (1972). The role of superoxide anion in the auto oxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* 247 (12): 3170-3175.
- Osipov, A.V., Kasheverov, I.E., Makarova, Y.V., Starkov, V.G., Vorontsova, O.V., Ziganshin, R.K.H., Andreeva, T.V., Serebryakova, M.V., Benoit, A., Hogg, R.C., Bertrand, D., Tsetlin, V.I. and Utkin, Y.N. (2008). Naturally occurring disulfide-bound dimers of three-fingered toxins: a paradigm for biological activity diversification. *Journal of Biological Chemistry*. 283:14571–14580.
- Reeks, T., Lavergne, V., Sumagar, K., Jones, A., Eivind, A.B., Nathan, U. and Fry, D.B. (2015). Deep venomomics of the *Pseudonaja* genus reveals inter- and intra-specific variation. *Journal of proteomics*.133:20–32.
- Rivel, M., Solano, D., Herrera, M., Vargas, M., Villalta, M., Segura, A., Arias, A.S., Leon, G. and Guti errez, J.M. (2016). Pathogenesis of dermonecrosis induced by venom of the spitting cobra, *Naja nigricollis*: an experimental study in mice. *Toxicon*, 119, 171–179.
- Sanhajariya, S., Duffull, S.B. and Isbister, G.K. (2018). Pharmacokinetics of Snake Venom. *Toxins (Basel)*. 7;10(2):73.
- Sani, I., Rabi`u Aliyu Umar, Sanusi Wara Hassan, Umar Zaki Faruq, Abubakar Abdulhamid, Fatima Bello and Isah Musa Fakai. (2019). Major Enzymes from Snake Venoms: Mechanisms of Action and Pharmacological Applications. *Asian Journal of Biological Sciences: Volume 12 (3)*: 396-403.
- Sies, H. (2017). Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biol.* 11, 613–619.
- Singh, N., Gupta, V.K., Kumar, A. and Sharma, B. (2017). Synergistic Effects of Heavy Metals and Pesticides in Living Systems. *Front. Chem.* 5, 70.
- Stevens-Truss, R., Messer, W.S and Jr Hinman, C.L. (1996). Heart and T-lymphocyte cell surfaces both exhibit positive cooperativity in binding a membrane-lytic toxin. *Journal of Membrane Biology*. 150(1):113–122.

- Tan, K.Y., Tan, C.H., Fung, S.Y. and Tan, N.H. (2015). Venomics, lethality and neutralization of *Naja kaouthia* (monocled cobra) venoms from three different geographical regions of southeast asia. *Journal of Proteomics*. 120, 105–125.
- Tasoulis, T. and Isbister, G.K. (2017). A review and database of snake venom proteomes. *Toxins* 9, 673290. Clinical toxicology research group, university of Newcastle, Newcastle 2298, Australia.
- Thom SR, Kang M, Fisher D. and Ischiropoulos H. (1997). Release of glutathione from erythrocytes and other markers of oxidative stress in carbon monoxide poisoning. *Am Physiol Society*; 82: 1424-1432.
- Vashney, R. and R.K. Kale, (1990). Effects of calmodulin antagonist. *Int. J. Radiat. Biol.*, 58: 733-743.