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# Effect of Intramuscular Administration of *Echis Ocellatus* Venom on some Organs of Wistar Rats Infected with *T.b. brucei* .

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## Abstract
*Trypanosomes are haemo-flagellate protozoans that inhabit the blood plasma, the lymph and various tissues of their host causing disease in both humans and animals which has been described as a complex debilitating and often fatal condition leading to death in severe cases. Effect of intramuscular administration of Echis ocellatus venom on kidney and liver of wistar rat infected with T.brucei brucei was carried out using 30 wister rats divided into six groups of five rats each. Group 1 were infected and administered 400ml of venom, group 2 were infected and administered 200ml of venom, group 3 were infected and administered 100ml of venom, group 4 were infected and administered 25ml venom, group 5 were infected and not administered venom and group 6 were infected and treated with Diminzene diaceturate. It was observed that at 25ml of snake venom administration into wistar rats, there was a significant rise (0.68± 0.01*) in the weight of the kidney as compared to the remaining dosage administered as shown in Table 1. The mean weight of liver which showed an increase in size as compared to the remaining dosages at 400ml (5.33± 0.04*), where the liver was slightly or thinly affected, indicating that high dosage of venom can be lethal to the liver of wistar rats as seen in Table 2. This study showed that both kidney and liver infected with T.b.brucei without administering snake venom and treated with Diminazene diaceturate gained more weight indicating hepatomegaly and nephritis, therefore, it can be concluded that Echis ocellatus venom has potential ingredient in combating the disease.*

**Keywords:** Diminazene aceturate, Organs, Snake venom, *Trypanosoma brucei brucei*, Wistar rats.

## INTRODUCTION
Trypanosomes are haemo-flagellate protozoans that inhabit the blood plasma, the lymph and various tissues of their host causing disease in both humans and animals (Ezeokonwo *et al.*, 2010) known as Trypanosomiasis. Most *trypanosome* species are transmitted cyclically via *Glossina* (tsetse flies) and mechanically by biting flies (*Tabanids and stomxys)* (Luckins & Dwinger, 2004), also experimental infections can also be established in the laboratory using mice, rats, guinea pigs and rabbit (Anosa , 1983; Cattand *et al.*, 2005; Fajinmi *et al.*, 2006). Infection in animal by one or more of the trypanosomes results in acute or chronic disease characterized by intermittent fever, anaemia, occasional diarrhoea, loss of appetite,

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weakness and coma leading to death in chronic conditions (Ikede & Losos 1972; Anosa, 1983; Bhatia *et al*., 2006), which has been described as a complex debilitating and often fatal condition which affects animals. Diminazene diaceturate is one of the most commonly used therapeutic drug for treatment of the disease in livestock in Sub-Saharan Africa (Geerts and Holmes, 1998). The main biochemical mechanism of the trypanocidal actions of Diminazene diaceturate is by binding to trypanosomal kinetoplast DNA (KDNA) in a non-intercalative manner through specific interactions with sites rich in adenine-thymine base pairs (Leach and Roberts, 1981).

*Echis ocellatus* is a highly venomous species of viper endemic to West Africa and commonly known as carpet snake found in dry regions. It is responsible for more fatalities than other African snakes. The venom of this snake is a compound of procoagulants, anticoagulants, hemmorraghins, nephrotoxins and necrotoxins which facilitates immobilization and digestion of prey (Buchot *et al*., 1994; Bottrall *et al*., 2010; Mattison *et al*., 2007; Reptile Venom Research, 2010), the main components of which are proteins and peptides (Casewell *et al*., 2013). Snake venoms are typically cytotoxic, neurotoxic and haemotoxic. The anticancer potential of cytotoxins has long been recognized (Estevão-Costa *et al*., 2018). However, the potential of snake venom in treatment of trypanosomiasis has not been established.

The role of liver and kidney in maintenance of life cannot be overemphasized, as the largest inter and major organ in the body, the liver metabolizes and detoxifies substances and also helps in the regeneration of body cells. The kidney on the other hand maintain health via its role in elimination of waste materials such as urea, creatinine, water and also maintenance of body electrolytes. Failure by these organs in part or full to perform these life function impairs metabolic activities and causes accumulation of waste materials,causing toxicity in the body which in severe conditions leads to death (Anene *et al.,* 2001, Sembullingam *et al.,* 2009, Arthur *et al*., 1998). The wistar rat was developed at the Wistar institute in 1906 for use in biological and medical research (Clause *et al.*, 1998). It thrives very well when restricted from drinking water, their liver and kidney have been speculated to have adaptive mechanism for ion and water homeostasis, thus good for laboratory purposes. Therefore, this study is aimed at establishing potential of snake venom as an anti-trypanosomal agent on liver and kidney of wistar rats infected with *T. b .brucei*.

## MATERIALS AND METHOD

### Experimental animals

A total of thirty (30) wistar rats were used for this study which were procured from the College of Health Science, Aminu Kano Teaching Hospital Laboratory Animal house. The rats were acclimatized for two weeks before the commencement of the experiment and were fed standard rat feed and water *ad libitum*. The donor rats infected with *T.b.brucei* were bled from the retro orbital plexus through the median canthus of the eye. A total of $1.0 \times 10^6$ trypanosomes suspended in 0.4ml of PBS were intramuscularly administered into 30 wistar rats which were assigned into six groups of five rats each respectively.

Group 1 were infected and administered 400ml of venom, group 2 were infected and administered 200ml of venom, group 3 were infected and administered 100ml of venom, group 4 were infected and administered 25ml venom, group 5 were infected and not administered venom and group 6 were infected and treated with Diminzene diaceturate.

The drug used for treatment was administered to the rats according to the manufacturer's instruction.

The rats were weighed using weighing balance and the animals were humanely sacrificed at regular intervals. A mid-ventral abdominal incision was made on each animal, the peritoneum reflected and the intestine displaced to gain access to the renal system. The kidney and the liver were then removed and weighed using precision scales HELMAC HM100.

**Statistical analysis**

All the data obtained for the organ weights are presented as mean ± SEM. Data were analyzed by the one-way analysis of variance (ANOVA) and the significance of differences between mean values computed for particular levels of experimental factors.

**RESULT**

The mean weight of kidney observed after 21 day post infection showed a significant change on wistar rats infected and administered 25ml of snake venom as compared to the remaining volumes of venom administered whereas group 5 showed a significant increase on the mean weight of kidney indicating the presence of nephritis, one of the signs of trypanosomiasis**.**

**Table 1: Mean weight (Kg) of Kidney of wistar rats infected with *T.b.brucei***

| IM/Day | 1 PI | 14 PI | 21 PI | MEAN VALUE |
|---|---|---|---|---|
| 400ml of SV | 0.41 ± 0.03 | 0.41± 0.03 | 0.41± 0.03 | 0.41± 0.03 |
| 200ml of SV | 0.49± 0.02 | 0.48 ± 0.02 | 0.47± 0.02 | 0.48± 0.02 |
| 100ml of SV | 0.41±0.03 | 0.43 ± 0.03 | 0.51 ± 0.03 | 0.45±0.03 |
| 25ml of SV | 0.68±0.01 | 0.68 ± 0.01 | 0.68± 0.01 | 0.68± 0.01* |
| Infected+ no venom | 1.90± 0.02 | 1.92 ± 0.02 | 1.95± 0.02 | 1.92± 0.02* |
| Infected + DA | 0.77 ± 0.01 | 0.77 ± 0.01 | 0.77± 0.01 | 0.77± 0.01 |

IM- intramuscular administration, SV- snake venom, DA- Diminazene diaceturate,
PI- Post infection, *- significant.

After 21 day post infection of T. b. brucei and administration of snake venom in volumes in the experimental animals, group 1 has a higher significant increase in weight of liver as compared to the remaining groups while group 5 which is the infected and untreated treated group showed a clear sign of hepatomegaly.

**Table 2: Mean weights (Kg) of liver of wistar rats infected with *T.b brucei***

| IM/Day | 1 PI | 14 PI | 21 PI | MEAN VALUE |
|---|---|---|---|---|
| 400ml of SV | 5.49 ± 0.04 | 5.30± 0.04 | 5.21± 0.04 | 5.33± 0.04* |
| 200ml of SV | 4.47± 0.02 | 4.40 ± 0.02 | 4.20± 0.02 | 4.36±0.02 |
| 100ml of SV | 4.31± 0.02 | 4.00±0.02 | 4.00±0.02 | 4.10± 0.02 |
| 25ml of SV | 4.20± 0.01 | 4.10± 0.01 | 4.10 ± 0.01 | 4.13± 0.01 |
| Infected + no venom | 7.60± 0.04 | 7.60 ± 0.04 | 7.65± 0.04 | 7.62± 0.04* |
| Infected + DA | 3.35 ± 0.02 | 3.53 ± 0.02 | 3.55 ± 0.02 | 3.48±0.02 |

IM- intramuscular administration, SV- snake venom, DA- Diminazene diaceturate,
PI- Post infection,*- significant.

## DISCUSSION

This study demonstrates the potential of snake venom on some organs of wistar rats infected with *Trypanosoma brucei brucei* thereby administering at different volumes for a period of 21 days therefore it is of upmost importance to observe the organ weight of experimental animals while undergoing chemotherapy studies, hence this proves that the kidney and liver of wistar rats showed significant change. Table 1 showed the mean value of the weight of kidney from Day 1 of post infection with snake venom at different volumes and the parasite. It was observed that at 25ml of snake venom administration into wistar rats, there was a significant rise in the weight of the kidney as compared to the remaining dosage administered. The weight of kidney infected and treated with diminazene diaceturate in this study agreed with the findings of Ajakaiye *et al*. (2019) and Ezejiofor *et al.* (2013). This weight of liver from wister rats infected with *T.b.brucei* and administering of *Echis ocellatus* venom at different dosages showed that at 400ml, the weight showed an increase in size as compared to the remaining dosages where the liver is slightly or thinly affected, therefore, high dosage of venom can be lethal to the liver of wistar rats. The weight obtained while treating with diminzene diaceturate agrees with the report of Ajakaiye *et al.* (2019) whereby the weight remains within the normal range. The mean weight of both organs were not at risk during the cause of the study following administration of snake venom as compared to infection with *T.b.brucei* which indicates that the volume of venom in the system of the experimental animal determines its toxicity therefore snake venom can be potential importance when the active components' is in use. It has been reported that minimal increase in organ weight without any microscopic lesion can be correlated with enzyme induction (Ramakrishna *et al.,*2015).

## CONCLUSION

This study shows that despite snake venom being poisonous in the body, it caused minimal to no damage to the kidney and liver of wister rats while trypanosomes lead to fatal damage on the organs of animals causing hepatomegaly and nephritis as observed from the study animals. Animal wellbeing and economic development can never be overemphasized, hence there is the need to tackle trypanosomes with peptides and proteins obtained from snake venom for its potential purposes.

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