Comparative Clinical Pathology
https://doi.org/10.1007/s00580-022-03341-5

ORIGINAL ARTICLE

Assessment of toxicity and anti-trypanosomal activities of toad venom in rat models

Yunusa O. Ibrahim[1] · **Musa B. Busari**[2] · **Michal A. Yisa**[3] · **Asmau N. Abubakar**[1] · **Fatima M. Madaki**[1] · **Ruqayyah S. Yusuf**[4]

Received: 18 November 2021 / Accepted: 18 March 2022
© The Author(s), under exclusive licence to Springer-Verlag London Ltd., part of Springer Nature 2022

Abstract

African trypanosomiasis is considered one of the neglected diseases leading to the death of thousands of people. The case of drug resistance, coupled with adverse side effects of the available drugs, warrants urgent alternative. In this study, acute toxicity, and prophylactic and suppressive anti-trypanosomal activities were carried out followed by sub-acute toxicity of the toad venom in rats. In acute toxicity test a total of nine Wistar rats were distributed into three groups and administered 10, 100 and 1000 mg/kg body weight (bw.) of the venom, respectively. Doses of 5, 10 and 20 mg/kg bw. of the venom were administered to the rats in their respective groups in the suppressive and prophylactic and sub-acute tests respectively, and 5 mg/kg bw. of diminazene aceturate was administered to the rats in their respective groups while 2 mL/kg bw. of normal saline was administered to the control and untreated groups. The LD_{50} of the venom was calculated to be 31.62 mg/kg bw. No significant differences ($p > 0.05$) were observed between the % suppression in the prophylactic and suppressive anti-trypanosomal activities of diminazene aceturate (100 and 99.80%) and the venom at 5 mg/kg bw. (100 and 98.80%) and 10 mg/kg bw. (100 and 100%), respectively. Significant increases ($p < 0.05$) in the levels of ALT, AST, ALP, creatinine, urea and uric acid were observed at dose of 20 mg/kg bw. of the venom. It is shown from this study that toad venom exhibits suppressive and prophylactic anti-trypanosomal activities with no biochemical and histological alterations at lower doses.

Keywords Toad venom · Acute toxicity · Prophylactic · Suppressive · Anti-trypanosomal activities

✉ Yunusa O. Ibrahim
yunusaibrahim1994@gmail.com

Musa B. Busari
busari.bola@futminna.edu.ng

Michal A. Yisa
michalyisa@gmail.com

Asmau N. Abubakar
Niwoye.asmau@futminna.edu.ng

Fatima M. Madaki
nmmadaki@gmail.com

Ruqayyah S. Yusuf
rusuf200@gmail.com

[1] Department of Biochemistry, Federal University of Technology, Niger State, PMB 65 Minna, Minna, Nigeria

[2] Oxidative Stress Related Diseases and Nanomedicine Research Group, Federal University of Technology, Niger State, PMB 65, MinnaMinna, Nigeria

[3] Department of Animal Biology, Federal University of Technology, Niger State, PMB 65 Minna, Minna, Nigeria

[4] Department of Biochemistry, Bauchi State University, Gadau, Bauchi State, Nigeria

Introduction

African trypanosomiasis is a neglected tropical disease caused by protozoan under the genus, *Trypanosoma*. African animal trypanosomiasis (AAT) also known as nagana in West Africa is mainly caused by three species of trypanosome which include *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosomas brucei brucei*, while human African trypanosomiasis (HAT) popularly called sleeping sickness is typically caused by *T. b. rhodesiense* and *T b. gambiense* (Awuah-Mensah et al. 2021). These different species of trypanosomes are harboured and transferred by tsetse flies, and the disease can be contracted by bite from these flies (Awuah-Mensah et al. 2021). Aside from the old Gboko endemic which still attracts focus till date, there have been a number of cases of the outbreak of the disease in several other communities in Nigeria (Mukhtar et al. 2017; Maimadu et al. 2018). The prevalence rates of this disease in different breeds of animals in Nigeria in the past decades have been estimated to be between 8.4 and 15.53%. The annual loss incurred in livestock farming

in Africa resulting from the disease is cost to be about 5 billion US dollars (Maimadu et al. 2018). In Africa, and most especially in Nigeria, trypanosomiasis is observed to be resurfacing as one of the major challenges in livestock farming affecting ruminants, equines, camels, to mention just a few (Fetene et al. 2021). The currently used anti-trypanosomal drugs used for the treatment of HAT include suramin, pentamidine, melarsoprol, eflorinithine, arsobal and mel B, while homidium, isometamidium and diminazene aceturate are used in treating AAT. Unfortunately, poor efficacy coupled with drug resistance and toxicity of these available drugs are limiting factors to achieving control over the disease (Kayode et al. 2020; Ibrahim et al. 2020).

Amphibians (toads) are considered one of the rich sources of bioactive compounds such as peptides, alkaloids, bufadienolides, biogenic amines and proteins with numerous pharmacological properties (Bordon et al. 2020). Although plants, marine invertebrates and reptiles are the most recognised sources of bioactive compounds, in the last two decades, amphibian secretions have attracted global attention owing to their pharmacological properties (Junior and Martins 2020). Several studies have shown these secretions to possess numerous pharmacological activities including antidiabetic (Udovychenko et al. 2020), antimicrobial (Udovychenko et al. 2019), cardiotonic (Sinhorin et al. 2020), immunomodulatory (Chai et al. 2021) and antiviral (Nikolaieva et al. 2018) activities, amongst others. These activities are traceable to the presence of the bioactive compounds in these secretions. Unfortunately, these secretions also contain numerous toxic compounds including bufadienolides, bufotoxins, bufotenine and dihydrobufotenine, which may cause serious physiological disorders as these secretions are produced for predation and protection of the amphibians (Brown 2020). Therefore, this study was aimed at evaluating the anti-trypanosomal activity as well as the toxicological effects of toad venom in Wistar rats.

Materials and methods

Collection and identification of toads

About forty (40) toads were obtained from swampy water lodge within the premise of Federal University of Technology, Bosso Campus, Minna, Niger State, Nigeria, with temperature and relative humidity of about 24.7–25.5 °C and 80–85%, respectively. The toads were collected around 5.00 am into a clean aerated plastic container. The identification of the toad was done by a senior Zoologist in the Department of Animal Biology, Federal University of Technology, Minna, Niger State, Nigeria, using a published proceeding of the Academy of Natural Sciences of Philadelphia (Halowell 1854), and an article in Zootaxa by Poynton et al. (2016). The species of the collected toads were identified as *Sclerophyrs maculata.*

Collection and maintenance of parasite (Trypanosoma brucei brucei)

The parasite was obtained from the Nigeria Institute for Trypanosomiasis Research (NITR), Vom, Plateau State. The parasite was maintained in the Animal House of the Department of Biochemistry, Federal University of Technology Minna, by continuous passaging of blood from donor rats (infected rats) into the uninfected ones until the commencement of the experiment (Abdulazeez et al. 2013).

Experimental animals

A total of sixty-five (65) male Wistar rats weighing 131 ± 5.00 g were purchased from the Department of Biochemistry Animal House, Federal University of Technology, Bosso Campus, Minna, Niger State, Nigeria. The animals were kept in standard cages at temperature of 27 ± 2 ℃ and relative humidity 46–53%. The animals were given free access to water and fed with pelletised commercial grower feed (Vital Feeds, Jos Nigeria) ad libitum. The experiment was performed following the review protocol (1997) of Canadian Council on Animal Care and Use guidelines. Ethical clearance number 000021 was given by FUT.MINNA/Nigeria Ethical Review Committee.

Methods

Extraction of the toad venom

The crude toad venom was obtained by manual compression of the parathyroid glands. The venom was collected into a sterile petri dish and lyophilized using freeze-dryer (LGJ-18). The lyophilized venom was kept inside refrigerator at 4 ℃ until the experiment commenced.

Acute toxicity test

The method of Lorke (1983) with slight modifications was used in the acute toxicity study of the toad venom. Briefly, nine rats were distributed into three groups of three rats each and administered doses of 10, 100 and 1000 mg/kg body weight of the toad venom, respectively. The animals were then observed for 24 h for sign of toxicity and mortality, after which the LD_{50} was calculated using the formula:

$$LD50 = \sqrt{(D0 - Dm)}$$

where D0 is the minimum tolerated dose and Dm is the minimum lethal dose.

Animals groupings for suppressive and repository anti-trypanosomal tests

There was implementation of a completely randomised designed experiment whereby animals of equal number, weight and sex were randomly distributed in the experimental groups in the same manner for both suppressive and prophylactic studies as indicated below:

Group 1 (normal control): administered 2 mL/kg body weight normal saline.
Group 2 (positive control): infected and administered 5 mg/kg body weight of diminazene aceturate.
Group 3 (negative control): infected and administered 2 mL/kg body weight normal saline.
Group 4: infected and administered 5 mg/kg body weight of the toad venom.
Group 5: infected and administered 10 mg/kg body weight of the toad venom.
Group 6: infected and administered 20 mg/kg body weight of the toad venom.

All animals in their respective groups were infected with 0.2 mL of buffered saline-diluted blood from the donor rat except for the normal control group. The body weight and packed cell volume (PCV) of the experimental rats were monitored before and after the experiment.

Determination of parasitemia

To determine the parasitemia of the experimental animals, blood was collected from the tail of each animal in each group on a microscope slide after diluting with buffered saline, then covered with cover slip and directly viewed under microscope at × 40 magnification. The 'rapid matching' method by Herbert and Lumsden (1976) was adopted for the estimation of total circulating parasites.

Determination of packed cell volume (PCV)

Blood from the tail of each animal in a group was collected into a capillary tube sealed with plastic sealant and centrifuged at 3000 rpm for twelve (12) minutes using haematocrit centrifuge. The PCV was then determined using haematocrit reader.

Evaluation of suppressive anti-trypanosomal activity of the toad venom

For the suppressive study, a total of 25 Wistar rats were infected with 0.2 mL of blood from parasitized donor rat with parasitemia level of about 1×10^6/mL. After two (2) hours of infection, the rats in their respective groups were treated as indicated above for four (4) consecutive days (Peters 1967) as reported by Georgewill et al. (2020). The parasitemia was determined on days 5, 10 and 15 post infection. The mean parasitemia was calculated for each treatment groups by comparing with the negative control, and the chemo-suppression was calculated as follows:

$$Chemo - suppression\ (\%) = \frac{A - B}{A} \times 100$$

where A is the mean parasitemia of negative control and B is the mean parasitemia of each treatment group.

Evaluation of prophylactic anti-trypanosomal activity (repository test) of the toad venom

The prophylactic anti-trypanosomal activity of the toad venom was evaluated following the method of Peters (1967) as reported by Georgewill et al. (2020) with slight modifications. Briefly, the rats in their respective groups were treated as indicated above for a period of four (4) days. On the 5th day, the rats were infected with 0.2 mL of blood from parasitized donor rat with parasitemia level of about 1×10^6/mL, and the parasitemia was determined on days 9, 15 and 20 post infection. The prophylactic activity of the venom was calculated as shown below:

$$Prophylactic\ activity\ (\%) = \frac{A - B}{A} \times 100$$

where A is the mean parasitemia of negative control and B is the mean parasitemia of each treatment group.

Animals grouping for the sub-acute toxicity testing

For the sub-acute toxicity test, a total of 20 male Wistar rats were distributed into four groups of five animals each and were administered different doses of the toad venom as indicated below:

Group 1 (control): administered 2 mL/kg bw. normal saline.
Group 2: administered 5 mg/kg bw. of the toad venom.
Group 3: administered 10 mg/kg bw. of the toad venom.
Group 4: administered 20 mg/kg bw. of the toad venom.

Sub-acute toxicity of the toad venom

For the sub-acute toxicity test, a total of 20 male Wistar rats were distributed into four groups of five animals each and were administered different doses of the toad venom as indicated above. The animals were administered different doses of the toad venom over a period of 28 days (OECD 2008). On the 29th day, the animals were euthanized by cardiac puncture under diethyl ether anaesthesia. Afterwards, the

blood, liver and kidney tissues were collected for biochemical and histological analyses respectively.

Biochemical analysis

Selected biochemical parameters were analysed using Agape commercial kits according to the following methods: alkaline phosphatase (Rec 1972), alanine and aspartate amino transferases (Reitman and Frankel 1957), urea (Kaplan et al. 1965), creatinine (Tietz et al. 1994) and uric acid (Tietz et al. 1994).

Histology of the liver and kidney

For the histological analysis of the liver and kidney tissues, a section of both tissues was fixed in neutral buffered formalin solution (10%) immediately after excision from the animals. The conventional paraffin embedding technique (dehydration by ascending grades of ethanol, using chloroform for clearing and embedding with paraffin wax at 60 °C) was employed in processing of the fixed tissues. Sections of 3–4 µm thick were obtained and stained with haematoxylin and eosin from prepared paraffin blocks. The processed tissue slices were studied under a light microscope (magnification × 40) (Busari et al. 2021).

Data analysis

Data obtained from the study were analysed using one-way analysis of variance (ANOVA) and the significant differences amongst groups were determined by Duncan multiple comparison post hoc test (Statistical Package for Social Sciences, version 22.0, SPSS Inc., Chicago, IL, USA). *p*-value less than 0.05 was considered significant. The data were expressed as mean ± standard error of mean of five replicates.

Table 1 Oral acute toxicity of the toad venom

Dosage (mg/kg bw.)	No. of animals used	Mortality	LD_{50}
10	3	3/0	31.62 mg/kg bw
100	3	3/3	
1000	3	3/3	

Results

In the oral acute toxicity study of the toad venom as shown in Table 1 below, mortality was observed at doses of 100 and 1000 mg/kg body weight whereas no mortality was recorded at 10 mg/kg body weight. As such, the LD_{50} of the toad venom was calculated to be 31.62 mg/kg body weight.

Effects of the sub-lethal doses of the toad venom on the body weight

Table 2 shows the effect of the sub-lethal doses of toad venom on the body weight changes in the experimental rats. Therein, an increase in the body weight of the rats treated with 5 and 10 mg//kg bw. as well as 5 mg/kg bw. diminazene aceturate was observed which was comparable to the control ($p > 0.05$). Contrarily, loss of body weight was observed in the rats administered 20 mg/kg bw. of the toad venom and the untreated group when compared to the control ($p < 0.05$). The changes in body weight observed in the suppressive test were similar to those in the repository test.

Effects of the sub-lethal doses of the toad venom on packed cell volume (PCV)

As shown in Table 3, the effects of the sub-lethal doses of the toad venom in rats for the suppressive and repository tests were observed to be similar. Groups administered 5

Table 2 Effect of sub-lethal doses of the toad venom on the body weight of *Trypanosome brucei*–infected rats

Treatment	Body weight (g)			
	Suppressive test		Repository test	
	Initial	Final	Initial	Final
5 mg/kg bw. TV	133.99 ± 2.69^a	141.18 ± 2.42^b	136.61 ± 3.80^a	139.13 ± 3.10^b
10 mg/kg bw. TV	138.10 ± 1.45^a	138.98 ± 1.93^b	141.03 ± 4.08^a	139.13 ± 4.10^b
20 mg/kg bw. TV	133.48 ± 2.57^a	122.72 ± 1.75^a	134.33 ± 2.83^a	125.13 ± 1.02^a
5 mg/kg bw. DA	135.52 ± 2.18^a	141.57 ± 2.07^b	137.25 ± 4.32^a	139.13 ± 3.58^b
Untreated	131.61 ± 3.37^a	120.36 ± 2.35^a	133.16 ± 3.53^a	124.13 ± 2.73^a
Control	132.94 ± 3.46^a	139.13 ± 5.02^b	131.94 ± 3.46^a	139.13 ± 5.02^b

Values are presented as mean ± standard error of mean (SEM) of five replicates

Values with different superscripts in a column are significantly different at $p < 0.05$

TV toad venom, *DA* diminazene aceturate

Table 3 Effect of sub-lethal doses of the toad venom on the PCV of *Trypanosome brucei*–infected rats

Treatment	PCV			
	Suppressive test		Repository test	
	Initial	Final	Initial	Final
5 mg/kg bw. TV	37.33 ± 1.20[a]	39.67 ± 1.45[b]	41.00 ± 2.08[a]	40.33 ± 1.45[c]
10 mg/kg bw. TV	38.33 ± 2.03[a]	41.00 ± 2.08[b]	38.67 ± 2.03[a]	40.00 ± 0.58[c]
20 mg/kg bw. TV	38.67 ± 2.30[a]	30.00 ± 0.58[a]	40.67 ± 2.19[a]	34.67 ± 2.67[b]
5 mg/kg bw DA	37.00 ± 1.73[a]	38.67 ± 0.33[b]	37.00 ± 1.73[a]	28.00 ± 1.15[a]
Untreated	38.00 ± 1.15[a]	28.67 ± 0.88[a]	39.33 ± 1.86[a]	40.67 ± 1.20[c]
Control	36.67 ± 1.76[a]	39.00 ± 0.58[b]	36.67 ± 1.76[a]	39.00 ± 1.76[c]

Values are presented as mean ± standard error of mean (SEM) of five replicates

Values with different superscripts in a column are significantly different at $p < 0.05$

TV toad venom, *DA* diminazene aceturate

and 10 mg/kg bw. of the toad venom respectively showed no reduction in PCV, similar to the group treated with 5 mg/kg bw. of diminazene aceturate. No significant difference ($p > 0.05$) was observed between these groups and the control group. On the other hand, the group administered 20 mg/kg bw. of the toad venom alongside the untreated group showed reduced PCV when compared to the control group ($p < 0.05$).

Suppressive anti-trypanosomal activities of the toad venom

Table 4 shows the results of the suppressive anti-trypanosomal activities of the sub-lethal doses of toad venom in *Trypanosome brucei*–infected rats. In this study, all groups of rats administered sub-lethal doses of toad venom showed substantial reduced parasitemia from day 5 to day 15 when compared to the untreated group. On days 5 and 15, percentage suppression of 100.00% and 100.00% were observed at doses of 5 and 10 mg/kg bw, respectively which were comparable to the group administered 5 mg/kg bw. diminazene aceturate (99.60%), while 74.88% was recorded for 20 mg/kg bw on days 5 and 15.

Repository anti-trypanosomal activity of the toad venom

Similar to the effect of the sub-lethal doses of the toad venom on the *T. brucei* observed in the suppressive test, all tested doses exhibited significant anti-trypanosomal activities but the activity of the venom at doses of 5 and 10 mg/kg bw. was higher than that of 20 mg/kg bw. The percentage prophylaxis recorded for doses of 5 and 10 mg/kg bw. and 5 mg/kg bw. diminazene aceturate was observed to show no significant difference. The percentage prophylaxis recorded for 5 and 10 mg/kg bw. on day 9 was 99.60% while 99.80% and 100.00% were respectively recorded on day 20. On the other hand, dose of 20 mg/kg bw. percentage prophylaxis of 74.88 and 49.88% on day 9 and 20, respectively (Table 5).

Table 4 Suppressive anti-trypanosomal activities of the toad venom in *Trypanosome brucei*–infected rats

Treatment	Parasitemia parasites/mL ($\times 10^6$) Day 5	Suppression (%)	Parasitemia Parasites/mL ($\times 10^6$) Day 10	Suppression (%)	Parasitemia Parasites/mL ($\times 10^6$/mL) Day 15	Suppression (%)
5 mg/kg bw. TV	0.51 ± 0.02[c]	99.19	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00
10 mg/kg bw. TV	0.25 ± 0.04[a]	99.60	0.00 ± 0.00	100.00	0.00 ± 0.00	100.00
20 mg/kg bw. TV	15.85 ± 0.18[d]	74.88	31.62 ± 0.12	74.88	31.62 ± 0.20	74.88
5 mg/kg bw. DA	0.25 ± 0.00[a]	99.60	0.00 ± 0.00	100.00	0.51 ± 0.05	99.19
Untreated	63.10 ± 0.98[e]	-	125.90 ± 2.34	-	251.20 ± 1.97	-

Values are presented as mean ± standard error of mean (SEM) of five replicates

Values with different superscripts in a column are significantly different at $p < 0.05$

TV toad venom, *DA* diminazene aceturate

Table 5 Repository anti-trypanosomal activities of the toad venom in *Trypanosome brucei*–infected rats

Treatment	Parasitemia parasites/mL (× 10^6) Day 9	Prophylaxis (%)	Parasitemia Parasites/mL (× 10^6) Day 15	Suppression (%)	Parasitemia Parasites/mL (× 10^6/mL) Day 20	Suppression (%)
5 mg/kg bw. TV	0.25 ± 0.00[a]	99.60	0.00 ± 0.00[a]	100.00	0.25 ± 0.06[b]	99.80
10 mg/kg bw. TV	0.25 ± 0.06[a]	99.60	0.00 ± 0.00[a]	100.00	0.00 ± 0.00[a]	100.00
20 mg/kg bw. TV	7.94 ± 0.18[b]	74.88	7.94 ± 0.23[b]	87.42	63.10 ± 0.89[c]	49.88
5 mg/kg bw. DA	0.25 ± 0.00[a]	99.60	0.00 ± 0.00[a]	100.00	0.25 ± 0.04[b]	99.80
Untreated	31.62 ± 0.45[c]	-	63.10 ± 0.39[c]	-	125.90 ± 1.56	-

Values are presented as mean ± standard error of mean (SEM) of five replicates

Values with different superscripts in a column are significantly different at $p < 0.05$

TV toad venom, *DA* diminazene aceturate

Effects of the sub-lethal doses of the toad venom on some markers of liver and kidney damage

As shown in Table 6, administration of sub-lethal doses of the toad venom at doses of 5 and 10 mg/kg bw. unaltered the analysed biochemical parameters when compared to the control group. However, at dose of 20 mg/kg bw. of the toad venom, alterations in these parameters were observed, and these alterations were significantly different ($p < 0.05$) from the control group. Administration of the toad venom at sub-lethal dose 20 mg/kg bw. resulted in elevated levels of AST, ALT, ALP, creatinine, urea and uric acid, respectively.

Histology of the liver and kidney of the Trypanosome brucei–infected rats administered sub-lethal doses of the toad venom

The histology of the liver of the *Trypanosome brucei*–infected rats is shown in Fig. 1 therein, it was observed that the toad venom at doses of 5 and 10 mg/kg bw. respectively caused no histological alterations as evident by the liver micrographs of the groups of rats administered these doses showing preserved hepatocytes, hepatocytes sinusoids and portal vein similar to the control group. However, administration of the toad venom at sub-lethal dose of 20 mg/kg bw. triggered histological alterations of the liver resulting in hepatocytes necrosis as well as congested hepatocytes sinusoids. Similarly, the toad venom at doses 5 and 10 mg/kg bw. respectively maintained the architecture of the kidney micrograph, and showing preserved glomerulus, distal convoluted tubules and capsular space of the tissue. The 20 mg/kg bw. dose of the toad venom altered kidney architecture resulting in distortion of the glomerulus, distal convoluted tubules and capsular space as shown in the kidney micrograph (Fig. 2).

Discussion

Trypanosomes induce anaemia by generating free radicals that distort the membrane of the red blood cells (RBCs) resulting in subsequent haemolysis of the RBCs (Jolayemi et al. 2020). Anaemia has been found to be a marked feature of trypanosomiasis and the severity is dependent on parasite load (Busari et al. 2014; Erin et al. 2020), and this

Table 6 Effects of the sub-lethal doses of the toad venom on some selected biochemical parameters in *Trypanosome brucei*–infected rats

Parameter	Dosage (mg/kg bw.)			
	5 mg/kg bw. TV	**10 mg/kg bw. TV**	**20 mg/kg bw. TV**	**Control**
AST	21.34 ± 1.23[a]	24.72 ± 1.68[a]	36.96 ± 1.67[b]	25.54 ± 1.40[a]
ALT	17.67 ± 1.34[a]	20.13 ± 1.75[a]	34.33 ± 1.80[b]	20.47 ± 1.53[a]
ALP	70.67 ± 2.76[a]	65.45 ± 1.51[a]	91.47 ± 1.48[b]	68.25 ± 1.29[a]
Creatinine	8.26 ± 0.27[a]	8.62 ± 0.20[a]	14.79 ± 1.18[b]	8.62 ± 0.30[a]
Urea	21.75 ± 1.27[a]	22.82 ± 1.42[a]	48.25 ± 1.40[b]	26.76 ± 2.03[a]
Uric acid	6.72 ± 0.25[a]	7.77 ± 0.46[a]	15.41 ± 1.01[b]	7.87 ± 0.29[a]

Values are presented as mean ± standard error of mean (SEM) of five replicates

Values with different superscripts in a column are significantly different at $p < 0.05$

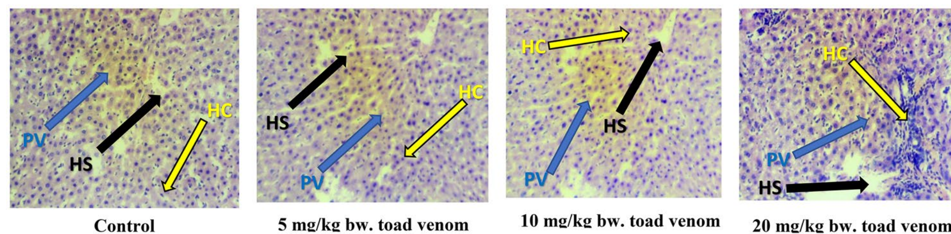

Fig. 1 Histology of the liver of male Wistar rats administered sub-lethal doses of toad venom (mg × 40). HS, hepatocytes sinusoids (black arrow); PV, portal vein (blue arrow); HC, hepatocytes (yellow arrow)

physiological disorder has been linked to loss of body weight as well as reduction in the PCV of the experimental rats (Goselle et al. 2020). A number of studies have shown that most trypanocidal drugs elicit their pharmacological activities by inhibiting essential enzymes of these parasites such as nucleotide-specific phosphodiesterases (Schoijet et al. 2020; Vo et al. 2020), ornithine decarboxylase (Boberg et al. 2021), S-adenosyl-l-methionine decarboxylase (Luisi and Carradori 2021), or suppressing the expression of genes such as ZC3H20 and RBP6 which are required for growth of procyclic form (PCF) and differentiation to metacyclic form (MCF) of the parasite, respectively (Ling et al. 2011; Kolev et al. 2012). Thus, the significant reduction in parasitemia observed in the rats administered 5 and 10 mg/kg bw. of the venom which was comparable to the rats treated with 5 mg/kg bw. diminazeneaceturate could be due to the ability of the bioactive principles (bufadienolides) contained in the venom (Rodriguez et al. 2021) to inhibit one or more of the abovementioned enzymes when the parasites surfaced in the blood or prevent the development of procyclic and metacyclic forms of the parasite by suppressing the expression of genes ZC3H20 and RBP6, respectively, thus completely eradicating the parasite from the bloodstream. Aregawi et al. (2021) reported that inability of the drug to reach the target sites in the parasites as a result of the ability of trypanosomes to shield from the toxic dose of administered drug could result in relapse of trypanosomes infections. Thence, the fascinating ability of the venom to keep the parasitemia in-check could be attributed to the ability of the bioactive principles of the venom to reach the target sites and permanently damaged the parasites. Furthermore, the relapse of the trypanosome on days 15 and 20 of the suppressive and repository tests observed at 5 mg/kg bw. of the venom and diminazene aceturate could be as a result of rapid degradation of the bioactive principles (Islam et al. 2021). Aregawi et al. (2021) also reported relapse of *T. evansi* infection at dose of 3.5 mg/kg bw. diminazene aceturate. The weight gain and improved PCV observed at doses of 5 and 10 mg/kg bw. of the venom could be as a result of the reduced parasitemia at these doses since the parasitemia determines the severity of anaemia which in turn negatively affects body weight and PCV. However, lower anti-trypanosomal activity coupled with weight loss and reduced PCV observed at 20 mg/kg bw. of the toad venom could be as a result of the hepatotoxic effect of the toad venom at this dose (as indicated in the biochemical and histological analyses) since liver is considered major organ responsible for the biotransformation of drugs.

Biochemical parameters (serum enzymes ALT, AST and ALP) and (serum creatinine, urea and uric acid) are some of the important indicators of liver and kidney damage, respectively. Elevated level of the serum enzymes implies damage to the liver hepatocellular membrane leading to extrusion of these enzymes into the blood (Yazar et al. 2020). Urea, creatinine and uric acid are major by-products excreted by the kidneys, and increased levels of these parameters are indicative of the kidney damage (Banaee et al. 2021). Thus, non-significant difference ($p > 0.05$) in the levels of markers of both liver and kidney damage between the control group and the groups administered 5 and 10 mg/kg bw. of the venom suggests that the venom at these doses is toxic to neither hepatocytes nor nephrocytes. However, administration of the venom at dose of 20 mg/kg bw. caused upsurge in the levels of these enzymes. This implies that the venom at this dose caused damage to the hepatocellular membrane which instigated the extrusion of the enzymes into the blood and reduced kidney excretion efficiency by reducing the amount of urea, creatinine and uric acid to be excreted from the blood thus leading to increased levels of these parameters in the serum. The toxicity observed at dose of 20 mg/kg bw. is not surprising because of the low LD_{50} (31.62 mg/kg bw.) of

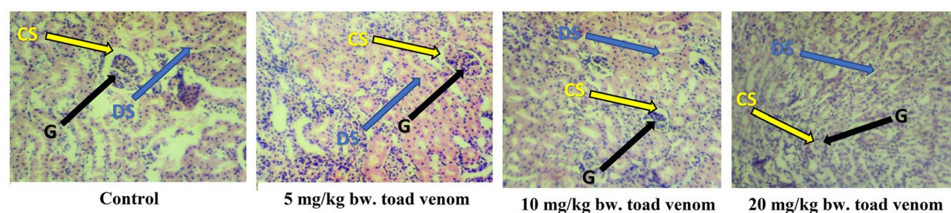

Fig. 2 Histology of the kidney of male Wistar rats administered sub-lethal doses of toad venom (mg × 40). G, glomerulus (black arrow); DC, distal convoluted tubules (blue arrow); CS, capsular space (yellow arrow)

the venom. The toxic properties of the venom at higher dose are in consonance with the reports of Oliveira et al. (2021) and Trakulsrichai et al. (2020), who reported toxic nature of toad venom at higher doses. Histological examinations of the liver and kidney tissues revealed that, at doses of 5 and 10 mg/kg bw., the venom triggered no histological abnormalities in liver and kidney micrographs of rats administered these doses showing preserved liver and kidney architectures similar to the control group. However, increase in dose up to 20 mg/kg bw. resulted in histological alterations such as hepatocyte necrosis and congestion of hepatocyte sinusoids of the liver, and distortion of glomerulus, convoluted tubules and capsular space of the kidney. This observation is in consonance with the findings of Chen and Huang (2013) who reported the same histological alterations in human exposed to high dose of toad venom. Again, the histological alterations observed at 20 mg/kg bw. could be traced to toxic nature of venom at higher doses (Chen and Huang 2013).

Conclusion

Conclusively from this study, toad venom at lower doses possesses anti-trypanosomal activities without toxic effects to the liver and kidney. However, with increase in dose, the anti-trypanosomal activity is reduced and it exhibits hepatotoxicity and nephrotoxicity. Toad venom can therefore be considered a potential drug lead for the development of drugs against *T. brucei* infections.

Author contribution All authors contributed to the concept and design of this study. Material preparation and data collection were performed by Yunusa O. Ibrahim and Michal A. Yisa, while data analysis was performed by Yunusa O. Ibrahim. The first draft of the manuscript was written by Yunusa O. Ibrahim and all authors commented on it. All authors read and approved the final manuscript.

Declarations

Conflict of interest The authors declare no competing interests.

References

Abdulazeez MA, Ibrahim AB, Edibo ZY, Sidali JO, Idris HO (2013) Anti-trypanosomal effect of *Peristrophe bicalyculata* extract on *Trypanosoma brucei brucei* infected rats. APJTB 3(7):523–531

Aregawi WG, Gutema F, Tesfaye J, Sorsa A, Megersa B, Teshome P, Ashenafi H (2021) Efficacy of diminazene diaceturate and isometamidium chloride hydrochloride for the treatment of Trypanosoma evansi in mice model. J Parasit Dis 45(1):131–136

Awuah-Mensah G, McDonald J, Steketee PC, Autheman D, Whipple S, D'Archivio S, Wickstead B (2021) Reliable scalable functional genetics in bloodstream-form Trypanosoma congolense in vitro and in vivo. PLoS Pathog 17(1):e1009224

Banaee M, Gholamhosseini A, Sureda A, Soltanian S, Fereidouni MS, Ibrahim ATA (2021) Effects of microplastic exposure on the blood biochemical parameters in the pond turtle (Emys orbicularis). Environ Sci Pollut Res 28(8):9221–9234

Boberg M, Cal M, Kaiser M, Jansson-Löfmark R, Mäser P, Ashton M (2021) Enantiospecific antitrypanosomal in vitro activity of eflornithine. PLoS Negl Trop Dis 15(7):e0009583

Bordon KDCF, Cologna CT, Fornari-Baldo EC, Pinheiro-Junior EL, Cerni FA, Amorim FG, Arantes EC (2020) From animal poisons and venoms to medicines: achievements challenges and perspectives in drug discovery. Front Pharmacol 11:1132

Busari MB, Ogbadoyi EO, Kabiru AY, Sani S (2014) Trypanocidal properties of aqueous leaf extract of *Morinda lucida*. Asian J Biol Sci 7(6):262–269

Busari MB, Hamzah RU, Muhammad HL, Yusuf RS, Madaki FM, Adeniyi JO, Ibrahim YO, Berinyuy EB (2021) Phenolic rich-extracts from Nauclea latifolia fruit restored Lead acetate-induced liver and kidney damaged in Wistar rats. Clin Phytoscience 7(1):1–10

Brown T (2020) A caution on handling Trachycephalus venulosus (Anura: Hylidae); toxic effects of skin secretion on human eyes. Herpetol Bull 152:28–29

Chai L, Yin C, Kamau PM, Luo L, Yang S, Lu X, Wang Y (2021) Toward an understanding of tree frog (Hyla japonica) for predator deterrence. Amino Acids 53(9):1405–1413

Chen L, Huang GZ (2013) Poisoning by toxic animals in China—18 autopsy case studies and a comprehensive literature review. Forensic Sci Int 232(1–3):e12–e23

Erin PJ, Orakpoghenor O, Sani NA, Adeke JT, Atata JA, Adamu S, Esievo KA (2020) Effects of acute experimental Trypanosoma brucei brucei infections on serum super oxide dismutase activities in relations to development of parasitaemia anaemia and leucopenia in Wistar rats. Comp Clin Pathol 29(2):311–316

Fetene E, Leta S, Regassa F, Büscher P (2021) Global distribution host range and prevalence of Trypanosoma vivax: a systematic review and meta-analysis. Parasit Vectors 14(1):1–20

Georgewill U, Harold M, Elias A (2020) Antiplasmodial activity of artemether-lumefantrine-tinidazole on Plasmodium berghei infected mice. Health Sci 2020

Goselle ON, Udoh SS, Ejete CO, Iruobe IA, Idoko S, Gyang AD, Matur BM (2020) Recorded marked changes in the haematological and immune responses of two non-transgenic rodents inoculated orally and intraperitoneally with Trypanosoma brucei brucei. Adv in Infect Dis 10(2):111–129

Hallowell E, Gibbons WP, Le Conte JL, Baird SF (1854) Descriptions of new reptiles from California; on a genus and species of serpent from Honduras presumed to be new; remarks on the geographical distribution of reptiles with descriptions of several species supposed to be new and corrections of former papers; description of four new species of viviparous fishes from Sacramento River and the Bay of San Francisco read before the California Academy of Natural Sciences May 15th 1854; synopsis of the Dermestidœ of the United States; synopsis. Proc Acad Nat Sci 91–120

Herbert WJ, Lumsden WHR (1976) Trypanosoma brucei: a rapid "matching" method for estimating the host's parasitemia. Exp Parasitol 40(3):427–431

Ibrahim Y, Busari M, Yusuf R, Hamzah R (2020) In vitro antioxidant activities of ethanol, ethyl acetate and n-hexane extracts of Mangifera indica leaves. Tanz J Sci 46(3):628–635

Islam T, Ganesan K, Xu B (2021) Insights into health-promoting effects of Jew's ear (Auricularia auricula-judae). Trends Food Sci Technol

Jolayemi KO, Mamman M, Sani D, Okoronkwo MO, Amaje J (2020) In vitro and in vivo changes observed in Trypanosoma brucei

brucei-infected rats treated with artesunate and/or diminazene aceturate. Sokoto J Vet Sci 14(4):211–220
Junior VH, Martins IA (2020) KAMBÔ: an Amazonian enigma. J Venom Res 10:13
Kaplan A, Chaney AL, Lynch RL, Meites S (1965) Urea nitrogen and urinary ammonia In Standard methods of clinical chemistry (5); 245–256.
Kayode OT, Lele CK, Kayode AA (2020) Trypanosomiasis: recent advances in strategies for control. Global J Infect Dis Clin Res 6(1):037–041
Kolev NG, Ramey-Butler K, Cross GA, Ullu E, Tschudi C (2012) Developmental progression to infectivity in Trypanosoma brucei triggered by an RNA-binding protein. Science 338(6112):1352–1353
Ling AS, Trotter JR, Hendriks EF (2011) A zinc finger protein TbZC3H20 stabilizes two developmentally regulated mRNAs in trypanosomes. J Biol Chem 286(23):20152–20162
Lorke D (1983) A new approach to practical acute toxicity testing. Arch Toxicol 54(4):275–287
Luisi G, Carradori S (2021) New compounds for the management of Trypanosoma brucei infection
Maimadu AA, Ogbu KI, Olabode MP, Waziri I, Salami CA, Ochai SO (2018) The prevalence of human African trypanosomosis in Maiduguri Nigeria. IOSR J Agri Vet Sci (IOSR-JAVS) 11(4)
Mukhtar Y, Abdu K, Maigari AK (2017) Efficacy of Anogeissus leiocarpus (DC) as potential therapeutic agent against trypanosomiasis diseases: a review. Int J Pharm Res 3(3):1–9
Nikolaieva I, Yu D, Oliinyk D, Oskyrko O, Marushchak O, Halenova T, Savchuk O (2018) Amphibian skin secretions: a potential source of proteolytic enzymes. Biotechnologia Acta 11(5)
Oliveira RS, Borges BT, Leal AP de Brum Vieira P, Silva DB, Hyslop S, Dal Belo CA (2021) Chemical and functional analyses of Rhinella icterica (Spix 1824) toad secretion screened on contractions of the heart and oviduct in Locusta migratoria. J Insect Physiol 129:104192
Organisation for Economic Co-operation and Development (2008) Test No 407: repeated dose 28-day oral toxicity study in rodents OECD Publishing
Peters W (1967) Rational Methods in the Search for Antimalarial Drugs Trans R Soc TropMed Hyg 1967(61):400–410
Poynton JC, Loader SP, Conradie W, Roedel MO, Liedtke HC (2016) Designation and description of a neotype of Sclerophrys maculata (Hallowell 1854) and reinstatement of S pusilla (Mertens 1937) (Amphibia: Anura: Bufonidae). Zootaxa 4098(1):73–94
Rec GSCC (1972) Colorimetric method for serum alkaline phosphatase determination. J Clin Bio 10(2):182
Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28(1):56–63
Rodriguez C, Ibáñez R, Mojica L, Ng M, Spadafora C, Durant-Archibold AA, Gutiérrez M (2021) Bufadienolides from the skin secretions of the Neotropical toad Rhinella alata (Anura: Bufonidae): antiprotozoal activity against Trypanosoma cruzi. Molecules 26(14):4217
Schoijet AC, Sternlieb T, Alonso GD (2020) Methods to Investigate signal transduction pathways in Trypanosoma cruzi: cyclic nucleotide phosphodiesterases assay protocols In Trypanosomatids. Humana New York NY (523–534)
Sinhorin AP, Kerkhoff J, Dall'Oglio EL, de Jesus Rodrigues D, de Vasconcelos LG, Sinhorin VDG, (2020) Chemical profile of the parotoid gland secretion of the Amazonian toad (Rhinella margaritifera). Toxicon 182:30–33
Tietz NW, Burtis CA, Shwood ER (1994) Tietz textbook of clinical chemistry Saunders
Trakulsrichai S, Chumvanichaya K, Sriapha C, Tongpoo A, Wananukul W (2020) Toad poisoning: clinical characteristics and outcomes. Ther Clin Risk Manag 16:1235
Udovychenko I, Oliynyk D, Dudkina J, Halenova T, Savchuk O (2019) Analysis of the Common spadefoot toad (Pelobates fuscus) skin secretions on the presence of the potential hemostasis system effectors. Bulletin of Taras Shevchenko National University of Kyiv-Biology 77(1):38–44
Udovychenko I, Oskyrko O, Marushchak O, Halenova T, Savchuk O (2020) Identification of biologically active fractions in the dermal secretions of the genus Bombina (Amphibia: Anura: Bombinatoridae). Acta Herpetologica 15(1):21–29
Vo KC, Günay-Esiyok Ö, Liem N, Gupta N (2020) The protozoan parasite Toxoplasma gondii encodes a gamut of phosphodiesterases during its lytic cycle in human cells. Comput Struct Biotechnol J 18:3861–3876
Yazar H, Kayacan Y, Ozdin M (2020) De Ritis ratio and biochemical parameters in COVID-19 patients. Arch Physiol Biochem 1–5

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.