



Advances in Research

12(6): 1-9, 2017; Article no.AIR.38539
ISSN: 2348-0394, NLM ID: 101666096

Toxicity of *Kigelia africana* Fruit in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author HMF designed the study, managed the literature searches, wrote the protocol and wrote the first draft of the manuscript. Authors AMEH and HEK guided the work. Author HMO managed the histopathology study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AIR/2017/38539

Editor(s):

(1) Farzana Khan Perveen, Founder Chairperson & Associate Professor, Department of Zoology, Shaheed Benazir Bhutto University (SBBU), Main Campus, Pakistan.

Reviewers:

(1) Daohong Chen, China.

(2) Veeravan Lekskulchai, Srinakharinwirot University, Thailand.

Complete Peer review History: <http://www.sciencedomain.org/review-history/22541>

Original Research Article

Received 30th November 2017
Accepted 17th December 2017
Published 1st January 2018

ABSTRACT

Aims: To evaluate the acute and chronic toxicity of the aqueous extract of *Kigelia africana* fruit in Wistar albino rats.

Methodology: The aqueous extract of *K. africana* fruit was administered orally to the rats in group 2, and 3 at a dose of 50, 500 mg/ kg body weight, respectively for test of chronic toxicity. Group 4 received 2000 mg/ kg for acute toxicity, whereas Group 1 was kept as a control. The animals were observed for clinical signs and mortality. The weights of animals were recorded at a weekly interval till the end of the experiment, and blood samples were collected weekly for hematological test and biochemical analysis. Livers and kidneys with pathological lesions were kept in 10% formalin for histopathological investigation.

Results: All animals survived in the four groups, and no mortality was recorded. The percentage of weight gain was highest in the control group. The hematological and biochemical parameters were not affected in group 2 and 3. White blood cells (WBC) were significantly ($P < 0.05$) increased in

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group 4 while red blood cells (RBC), hemoglobin (Hb) and packed cell volume (PCV) were significantly ($P < 0.05$) decreased. Total protein, and albumin were significantly ($P < 0.05$) decreased; cholesterol, creatinine and Urea were significantly ($P < 0.05$) increased. Bilirubin was not affected in all groups. Alanine Transaminase, ALT (Glutamate Pyruvate Transaminase GPT), Aspartate Transaminase, AST (Glutamate Oxaloacetate Transaminase, GOT) and ALP (Alkaline phosphatase) were significantly elevated.

Conclusion: The highest dose of the aqueous extract of *K. africana* fruit may have some hepatorenal toxic effects.

Keywords: *Kigelia africana*; toxicity; fruit extract; rats.

1. INTRODUCTION

The objective of plant toxicity testing is to elucidate the toxic properties of the plant part. The toxicity of *K. africana* fruit extract is necessary since this has not been previously done in depth.

Kigelia africana has been used traditionally as a remedy for a number of diseases in Africa [1]. Most commonly traditional healers used it to treat a wide range of skin ailments like, fungal infections, boils, psoriasis, and eczema. It also has internal application including the treatment of dysentery, ring worm, tape worm, postpartum hemorrhage, malaria, diabetes and toothache [1]. Moreover, in the folk medicine, the fruits of the plant are used as dressing of ulcers, purgative and increase the flow of milk in lactating women. The same authors reported that the pharmacological activities of the plant include antibacterial and antifungal, antineoplastic, analgesic and anti-inflammatory, anti-malarial, central nervous system stimulant, antiprotozoal and anti-diarrheal. Only scanty information is available regarding the toxicity of *K. africana* fruit.

In Sudan, *K. africana* has been investigated as antimicrobial [2,3], and antitubercular [4]. The *in vitro* screening for antitubercular activity showed slight cytotoxicity. Accordingly, to assess its activity *in vivo*, screening for its toxicity is needed. The present work investigates some clinical, hematological, biochemical and histopathological effects produced by the experimental use of the aqueous extract of *K. africana* fruit.

Many plants contain a number of chemical constituents and are employed for different medicinal purposes. However, over-dosage of plant products containing medicinal compounds may cause toxic reaction when introduced into human, animals and birds [5]. The toxic chemicals produced by plants (Phytotoxins)

include alkaloids, glycosides, sulphur, phenol, tannin, proteins, and enzyme inhibitors [6]. Toxins have direct and indirect mechanisms of actions on the most frequently induced organs: liver, kidney, brain, lung, intestine and others. The mechanisms of actions include direct damage of tissue, effect on function and genetic defect [7].

Phytochemical analysis of the aqueous extract of *K. africana* fruit showed alkaloids, flavonoids, saponins, tannins, phenolics, water soluble phenolics, water insoluble phenolics [8,9].

2. MATERIALS AND METHODS

2.1 The Plant

Kigelia africana (Lam.) Benth. in Hook., F1. Nigr.: 463 (1849) - belongs to the family Bignoniaceae - is widely distributed in the South, Central and West Africa. It is known in Sudan as Abu Shoutour, Umm Shoutour, Umm Mashatour; and in England as African Sausage tree or Cucumber because of its huge fruits (average length 0.6 m and weight 4.0 kg), which hangs from long fibrous stalks.

2.1.1 Plant collection

K. africana (Lam.) fruits were collected from the The Eastern Nuba Mountains. The plant part was identified and authenticated at the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan. The voucher specimen has been deposited in the herbarium museum of the Institute. The fruit was cut into small pieces; air dried in the shade, coarsely powdered and kept in polythene bags at room temperature.

2.2 Animals

Clinically normal, twenty four male Wistar albino rats were brought from Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan,

and kept in metal cages. They were left to adapt for one week prior to the start of the experiment. The rats were fed with a standard diet which is manufactured commercially for poultry (Layers) and vegetables. Feed and water were provided *ad libitum*. This study has been approved by the Ethical Approval No. EA / 0016 /2017, The Sudan Veterinary Council, Ministry of Cabinet, Republic of The Sudan.

2.3 Preparation of Aqueous Extract

The plant aqueous extract was prepared as described previously [10]. Hot distilled water (500 ml) was added to 100 g of the coarsely powdered plant fruit and left till cooled down with continuous stirring at room temperature. The extract was then filtered through What Man No. 1 filter paper, and kept at -10°C overnight. The frozen extract was transferred to the freeze drier (Trivac, U.S.A.) till the ice was removed, and powdered extract was obtained. The yield percentage of the extract was calculated as below:

$$\text{Yield percentage} = (\text{Weight of extract obtained}) / (\text{Weight of plant sample}) \times 100$$

The required weight of the aqueous extract for each group was calculated according to the dose; dissolved in 6 ml of distilled water. The volume of the extract administered to each animal based on the body weight.

2.4 Experimental Designs

Twenty four male Wistar albino rats weighing (103- 123 g) were divided into four groups, each of 6 rats. Group 4 was used for testing of acute toxicity, groups 2 and 3 for chronic toxicity, and group 1 was kept as a control. The extract was given at one of the fixed dose level (50, 500 and 2000 mg/kg).

2.4.1 Screening of aqueous extract of *K. africana* fruit for acute and chronic toxicity

A single dose of 2000 mg/kg was administered orally to the rats in group 4 for acute toxicity. For chronic toxicity, groups 2 and 3 were given the extract at a dose of 50 and 500 mg/ kg/ day, respectively, for four weeks. Group 1 was kept as a control.

Clinical observations and mortality rates were reported daily for acute and chronic toxicity. The weights of rats were recorded at the day of dosing, at weekly intervals thereafter, and at the time of death or when the animals were sacrificed.

2.5 Blood Collection

Blood samples were collected weekly-starting from week zero-from the orbital sinus of rat's eye - in Ethylene diamine tetra acetic acid (EDTA) vacutainers for hematological examination of White blood cells (WBC), Red blood cells (RBC), Hemoglobin (Hb) and Packed cell volume (PCV) using Sysmex Haematology System KN-21N/Germany, and plain vacutainers for serum analysis (Total protein, Albumin, Cholesterol, Bilirubin, Urea, Creatinine ALT, AST, ALP) using Sysmex Biochemistry System / Germany. The procedures were carried out as described in the manuals of the automated machines.

2.6 Pathological Examination

Rats in group 1, 2, 3, and 4 were sacrificed at the end of the experiment. The postmortem findings were recorded and specimens of normal liver and kidney, and liver and kidney with pathological lesions were fixed in 10% neutral buffered formalin and processed for histopathological examination.

2.7 Statistical Analysis

The data collected during the study were analyzed using the computer program SPSS version 21. The statistical analysis was done using ANOVA. The data are expressed as mean \pm SD. The results with $P < 0.05$ were considered significant.

3. RESULTS

3.1 Yield Percentage

The yield percentage (w/w) of aqueous extract of *K. africana* fruit was 17.02%.

3.2 Effect of *K. africana* Extract on Mortality of Rats

The extract was well tolerated by the animals as no signs of toxicity or mortality were observed after oral administration of the doses 50, 500 and 2000 mg/kg body weight to group 2, 3 and 4, respectively.

3.3 Weight Changes

The body weights of rats in group 1, 2, 3, and 4 were significantly ($P < 0.05$) increased (Fig. 1). The highest percentage of weight gain was in group 1 whilst the lowest in group 4 (Table 1).

3.4 Hematological Changes

The hematological changes on blood of rats given aqueous extract of *K. africana* fruit was presented (Table 2). WBC, RBC, Hb and PCV were not affected in group 2 and 3 but significantly ($P < 0.05$) changed in groups 4.

3.5 Biochemical Changes

The results of the toxicological effects on the biochemical parameters were summarized in Table 3. Oral administration of the aqueous extract of *K. africana* at doses of 50 mg / kg (group 2) and 500 mg/ kg (group 3) had no

effect. However, a dose of 2000 mg/ kg significantly ($P < 0.05$) altered all the biochemical parameters except billirubin.

3.6 Histopathological Findings

Gross anatomy of rats in group 1, 2 and 3 showed normal liver and kidney. Acute toxicity (Group 4) revealed histopathological changes in liver and kidney of rats. The liver was characterized by presence of vacuoles in hepatocytes cytoplasm, necrosis and dissociation of hepatocytes with loss of rod arrangement of hepatocytes (White arrow), and dilatation of sinusoids (Fig. 1B) compared with the control (Fig 1A). The kidney showed shrinking and segmentation of glomerular tuft. In some glomuli complete absence of glomular tuft, dilatation and necrosis of convoluted tubules and congestion (Fig. 2B) compared with control which revealed adequate preservation of tubular structures with the presence of glomerular tuft (Fig. 2A).

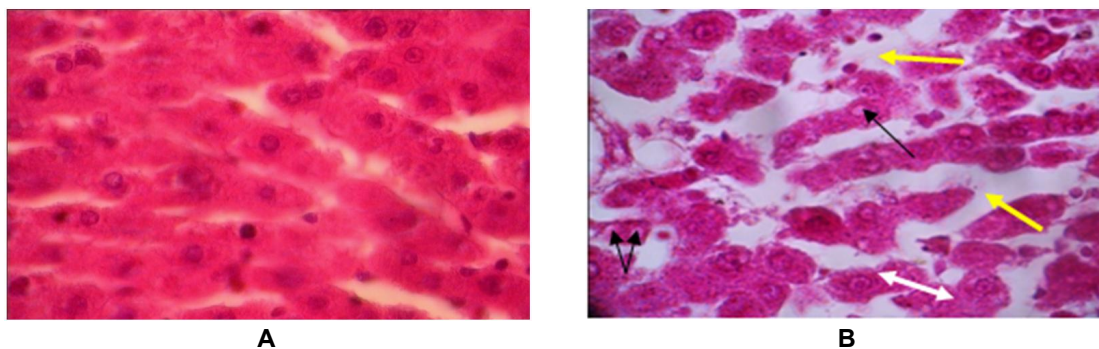


Fig. 1. Section of rat Liver (A) Normal control in group 1. (B) After given aqueous extract of *K. africana* fruit at a dose of 2000 mg/ kg (group 4) showed Presence of vacuoles in hepatocytes cytoplasm (Black arrows), necrosis and dissociation of hepatocytes with loss of rod arrangement of hepatocytes (White arrow), and dilatation of sinusoids (yellow arrows), H&E ($\times 40$)

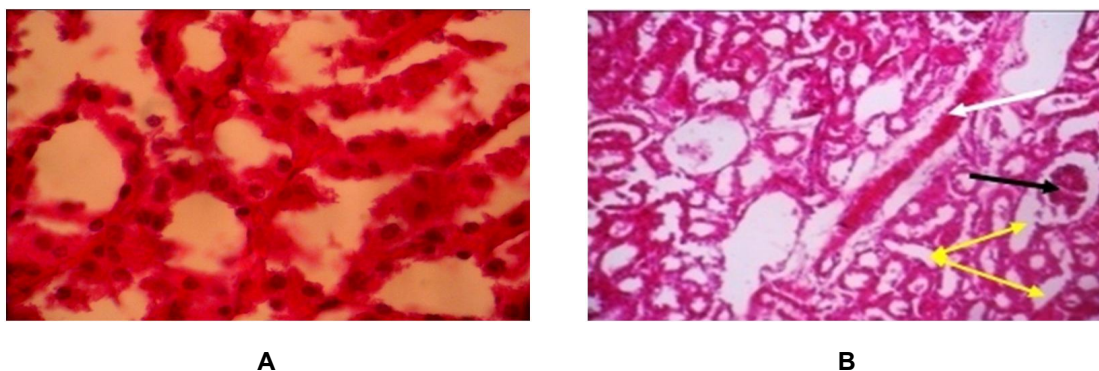


Fig. 2. Section of rat Kidney (A) Normal control in group 1. (B) After dosing of 2000 mg/ kg aqueous extract of *K. africana* fruit (group 4) shrinking and segmentation of glomerular tuft (Black arrow). In some glomuli complete absence of glomular tuft, dilatation and necrosis of convoluted tubules (Yellow arrows), and congestion (White arrow) were observed, H & E ($\times 40$)

Table 1. Percentage of weight gain of rats given aqueous extract of *Kigelia africana* fruit

Group No.	Dose (mg/kg)	Mean weight of rats (g) Week number					Weight gain (g)	Weight gain (%)
		0	1	2	3	4		
1	0	114.52±2.64	124.03±2.16*	133.19±2.59*	142.19±1.78*	153.70±2.24*	36.83	32.16
2	50	123.68±2.88	133.85±2.27*	143.53±2.21*	152.37±3.19*	161.54±3.39*	37.86	30.61
3	500	122.21±2.11	122.52±1.68*	132.86±1.94*	142.87±1.98*	153.70±2.24*	31.49	25.77
4	2000	103.14±2.41	111.16±1.35*	112.49±2.15*	115.82±2.81*	-	12.68	12.29

The data presented as Mean ± SD, *P < 0.05 is significantly different from the control, n= 6

Table 2. Hematological changes on the blood of rats given aqueous extract of *K. africana* fruit

Group No.	Week No.	Dose (mg/kg)	WBC ($\times 10^3 \text{mm}^3$)	RBC ($\times 10^6 \text{mm}^3$)	Hb (g/dl)	PCV (%)
1	0	0	5.90±0.14	6.32±0.08	11.68±0.19	37.50±0.40
	1		5.90±0.09	6.35±0.10	11.72±0.21	37.53±0.39
	2		5.92±0.08	6.34±0.07	11.72±0.21	37.55±0.39
	3		5.93±0.10	6.38±0.07	11.73±0.15	37.57±0.42
	4		5.92±0.15	6.37±0.08	11.73±0.15	37.57±0.43
2	0	50	5.90±0.18	6.33±0.14	11.57±0.16	37.40±0.86
	1		5.92±0.16	6.35±0.10	11.58±0.15	37.40±0.64
	2		5.92±0.16	6.35±0.10	11.58±0.15	37.47±0.82
	3		5.92±0.16	6.35±0.10	11.58±0.15	37.48±0.83
	4		5.93±0.15	6.32±0.12	11.57±0.16	37.48±0.83
3	0	500	5.85±0.10	6.55±0.16	11.73±0.28	37.85±0.26
	1		5.87±0.10	6.37±0.16	11.55±0.23	37.68±0.26
	2		5.87±0.10	6.33±0.19	11.43±0.21	37.55±0.26
	3		5.93±0.10	6.30±0.14	11.42±0.19	37.48±0.28
	4		6.03±0.10	6.27±0.12	11.33±0.21	37.43±0.30
4	0	2000	6.50±0.32	7.22±0.21	12.00±0.24	38.87±0.62
	1		9.08±0.41*	4.53±0.31*	8.82±0.51*	36.78±0.76*
	2		9.27±0.40*	4.40±0.29*	8.63±0.54*	36.57±0.77*
	3		9.42±0.41*	4.27±0.34*	8.48±0.55*	36.33±0.56*
	4		-	-	-	-

The data expressed as Mean ± SD, *P < 0.05 is significantly different from control by ANOVA, n= 6

Table 3. Biochemical changes on blood of rats after oral administration of the aqueous extract of *Kigelia africana* fruit

Group No. / Dose (mg/kg)	Week No.	Total protein (g/dl)	Albumin (g/dl)	Cholesterol (mg/dl)	Bilirubin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	ALT U/L	AST U/L	ALP U/L
1 (0)	0	6.15±0.18	3.53±0.16	43.00±0.42	0.10±0.00	14.00±0.07	0.58±0.08	18.33±0.82	13.00±0.89	53.00±1.41
	1	6.39±0.18	3.55±0.31	43.00±1.10	0.10±0.00	14.68±0.23	0.53±0.05	18.33±0.82	13.05±0.90	53.00±1.41
	2	6.46±0.19	3.65±0.23	43.17±0.75	0.10±0.00	14.68±0.32	0.53±0.05	18.42±0.83	13.05±0.90	53.00±1.67
	3	6.57±0.26	3.73±0.23	43.17±0.75	0.10±0.00	14.70±0.28	0.58±0.00	18.42±0.83	13.08±0.94	53.17±1.67
	4	6.67±0.16	3.80±0.17	43.33±0.52	0.10±0.00	14.70±0.26	0.60±0.00	18.43±0.80	13.08±0.94	53.17±1.67
2 (50)	0	6.43±0.08	3.60±0.11	43.30±1.03	0.10±0.00	14.75±0.10	0.58±0.08	18.50±0.48	12.33±0.36	54.17±0.75
	1	6.47±0.08	3.63±0.08	43.50±0.84	0.10±0.00	14.75±0.10	0.58±0.08	18.50±0.47	12.35±0.34	54.17±0.75
	2	6.48±0.06	3.67±0.08	43.50±0.84	0.10±0.00	14.80±0.09	0.60±0.00	18.60±0.47	12.35±0.15	54.33±0.52
	3	6.48±0.06	3.67±0.05	43.67±1.03	0.10±0.00	14.80±0.09	0.67±0.05	18.60±0.43	12.37±0.33	54.33±0.52
	4	6.52±0.06	3.75±0.08	43.83±1.17	0.10±0.00	14.80±0.00	0.67±0.05	18.67±0.48	12.37±0.33	54.50±0.55
3 (500)	0	6.55±0.19	3.70±0.14	45.67±1.03	0.13±0.05	15.17±0.33	0.57±0.08	17.00±0.14	11.07±0.27	50.00±2.33
	1	6.48±0.15	3.57±0.16	46.33±0.82	0.13±0.05	15.40±0.26	0.60±0.00	17.17±0.14	11.20±0.24	50.50±1.87
	2	6.35±0.14	3.42±0.13	46.67±0.52	0.13±0.05	15.48±0.29	0.60±0.00	17.42±0.08	11.50±0.29	50.50±2.17
	3	6.22±0.13	3.35±0.15	46.83±0.75	0.13±0.05	15.58±0.25	0.63±0.05	17.72±0.28	11.58±0.28	50.83±1.94
	4	6.10±0.13	3.20±0.14	46.83±1.17	0.13±0.05	15.80±0.24	0.63±0.05	17.80±0.24	11.67±0.26	50.83±1.72
4 (2000)	0	6.80±0.07	3.89±0.16	44.50±1.05	0.10±0.00	15.70±0.18	0.52±0.04	18.24±0.24	12.00±0.18	56.5±1.05
	1	4.62±0.12*	2.40±0.24*	47.60±0.47*	0.12±0.04	19.32±0.25*	0.87±0.05*	23.00±0.89*	19.08±0.29*	65.00±1.41*
	2	4.57±0.11*	2.32±0.26*	47.78±0.48*	0.15±0.05	19.40±0.14*	0.97±0.05*	30.00±0.53*	25.00±0.22*	69.15±0.63*
	3	4.52±0.10*	2.18±0.23*	47.93±0.46*	0.15±0.05	19.60±0.19*	1.03±0.05*	30.13±0.55*	25.98±0.54*	69.35±0.61*
	4	-	-	-	-	-	-	-	-	-

The data presented as Means ± SD, P<0.05: significantly different from control by ANOVA, n=6

4. DISCUSSION

The therapeutic activity of the plant *K. africana* and the likelihood of its traditional use, acute and chronic toxicity tests were done in the plant extract.

The aqueous extract is most commonly used in African traditional medicine. Though the plant *K. africana* is a rich source of many chemical compounds as it is known in Bignoniaceae [11]. This plant is not extremely toxic one. Organic solvent extracts have more toxic compounds like naphthoquinone, coumarine and iridoids. But water extract may be favorable for some applications like such ones on the skin [12].

The combined effects of physiological and chemical factors in the metabolism system of animals could lead to increase in WBC [13]. This could be the case with test rats in the present study. WBC in group and 4 were significantly ($P < 0.05$) increased against those of the control. Increased number of WBC indicated inflammation as seen in histopathological investigation. The mechanism of WBC and its components are defensive against foreign substances. Decreased RBC, Hb and PCV indicated decreased RBC production or increased RBC destruction.

Decrease of serum albumin could be indicative of impaired liver excretory and synthetic function. The observed increase ($P < 0.05$) of serum urea and creatinine in group 4 suggest renal malfunction [14].

Primary and secondary hepatic disease can cause an elevation of both ALT and AST [15]. Elevated transaminases are suggestive of liver necrosis [16].

The results of acute toxicity in this study was supported by [17] who reported that the extract administered orally at a dose of 2000 mg/kg was well tolerate by the animals as no signs of toxicity like restless, dizziness or seizures were observed. Furthermore, oral acute toxicity assay did not show any mortality at 5000 mg/ kg of the methanolic extract of *K. africana* bark, leaf and fruit [18]. However, it was found that the acute toxicity of the fruit ethanolic extract administered intra peritoneally to female Swiss mice at a dose of 1600 mg/ kg had a LD_{50} of 1.3 g/ kg [19]. Additionally, aqueous extract of *K. africana* fruit displayed significant toxicity towards *Artemia fanciscana naupli* with LD_{50} of 477 μ g/ ml [8]. In

the present study, the oral administration of the fruit extract and its passage along the digestive system process probably decreased the toxicity of the extract by enzymatic and metabolic pathways during progression in the digestive tract [20].

On the other hand, chronic toxicity test of *K. africana* fruit indicated that doses of 50, 500 mg/ kg given to rats in group 2, 3, respectively had no toxic effect and no mortality. This result was confirmed by the previous study of sub chronic toxicity of aqueous extract of *K. africana* fruit which administered orally to Wistar albino rats at doses of 100 and 500 mg/kg for 30 days. No organ specific toxicity was recorded; hematological (Hb, RBC) and biochemical parameters (albumin, creatinin, AST, ALT, ALP) were not affected [12]. In sub acute toxicity the methanolic extract of *K. africana* bark, leaf and fruit was safe and had no adverse effect on hematology of rats at doses of 100, 500 and 1000 mg/ kg [18]. However, the present finding was in contrast with other author [21] who found that the aqueous extract of *K. africana* fruit given orally to Wistar rats at a dose of 400 mg/ kg/ day was toxic but not fatal. This may be due to the different seasons of collection and the stage of ripeness of the fruits.

5. CONCLUSION

The findings revealed that the aqueous extract of *K. africana* fruit at low doses was safety, but high dose may have hepatorenal toxic effects. Further work is needed for determination of LD_{50} and LD_{99} . Phytochemical analysis and mechanisms of actions are recommended to define the toxic compounds that may exist.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws were applicable. The study has been approved by the Ethical Approval No. EA / 0016 / 2017, The Sudan Veterinary Council, Ministry of Cabinet, Republic of the Sudan.

ACKNOWLEDGEMENT

The authors acknowledge the Central Veterinary Research Laboratory for permission to publish

this article. Our thanks extended to Prof. Faiza Ahmed Omer, the technologist Omima Hassan Mohammed and the technician Mohamed Nageeb Ahmed at the Department of Pathology and Diagnosis for postmorteum, and histopathology technique, respectively. Great thank to the technologist Faiza Fadul at the Department of Tse Tse and Trypanosomes Control for statistical analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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