Sjournals
Scientific Journals

Scientific Journal of Veterinary Advance (2016) 5(7) 105-113
ISSN 2322-1879
doi: 10.14196/sjva.v5i7.2179

CODEN (USA): SJVAAS

Contents lists available at Sjournals
Scientific Journal of **Veterinary Advances**
Journal homepage: www.Sjournals.com

Original article

Histopathology of *Trypanosoma brucei brucei* infected red Sokoto goats experimentally infected and treated with crude ethanolic extract of *Terminalia catappa*

Jocelyn Iyowamhe Agbanu*, Mathew Chika Emere, Yahaya Abdullahi Umar
Department of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria.

*Corresponding author; Department of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria.

ARTICLE INFO

Article history,
Received 11 June 2016
Accepted 10 July 2016
Available online 17 July 2016
iThenticate screening 14 June 2016
English editing 08 July 2016
Quality control 14 July 2016

Keywords,
Crude ethanolic extract
Histopathology
Terminalia catappa extract
Trypanosoma brucei brucei
Red Sokoto goats

ABSTRACT

The trypanocidal activity of crude ethanolic extract of *Terminalia catappa* stem bark was investigated on red sokoto goats experimentally infected with *Trypanosoma brucei brucei.* 15 male goats weighing between 8kg to 10kg were used in this experiment, randomly grouped into 5 groups with three goats per group. Goats in all the 5 groups were infected intravenously with *T.b. brucei* and after demonstration of parasitaemia Groups 1 to 4 were administered crude ethanolic extract of *T. catappa* as follows: 50mg/kg orally to group 1, 100mg/kg orally to group 2, 50mg/kg intraperitoneally to group 3 and 100mg/kg intraperitoneally to group 4 while group 5 served as untreated control. All treatments lasted for 7 days. Phytochemical analysis revealed the presence of alkaloids, saponins, terpenoids and steroid. The histopathological findings did not reveal any effect of the extracts on the hearts of all *T. catappa* treated groups, although, lesions were observed on the spleen, liver, kidney and lung of both treated and untreated (control) group which could be as a result of the infection.

1. Introduction

Trypanosomiasis is a disease caused by trypanosomes which are protozoan parasites infecting a variety of domestic and wild animals as well as humans. Human African Trypanosomosis (HAT), is caused by *Trypanosoma brucei gambiense* and *Trypanosoma rhodesiense* which is commonly called sleeping sickness, while *Trypanosoma brucei brucei,* is responsible for Animal Africa Trypanosomiasis (AAT), or Nagana in animals. In Africa, trypanosomosis is transmitted by tsetse fly of the Genus *Glossina* which is found in 37 countries of Africa (Denbarga et al., 2012). In these countries the disease poses a significant health challenge to humans and livestock (WHO, 2012). The widespread occurrence of Trypanosomosis largely accounts for Africa's low livestock productivity and it is responsible for reducing the source of animal protein as well as contributing to food insecurity in the region (Malafaia and Talvani, 2011). Trypanosomiasis is transmitted biologically through the bite of a tsetse fly (*Glossina spp*.) but may also be transmitted through fomites and mechanical vectors including surgical instruments, needles, syringe and various biting flies (*Tabanids)*. Some species of trypanosomes are reported to be transferred between mammals through exchange of body fluids, such as in blood transfusion or sexual contact (Rocha et al., 2004).

Infection in humans is characterized within a few weeks by swollen lymph glands, aching muscles and joints, headaches and irritability. The second phase of the disease is characterized by involvement of the central nervous system with extensive neurological effects which, if not treated may cause changes in personality, alteration of the biological clock (the cardiac rhythm), confusion, slurred speech, seizures and difficulty in walking and talking and finally, terminating in death if not treated (Mare, 2008). However, Nagana which results due to infection with *Trypanosoma brucei brucei* becomes apparent in infected animals in about 7-10 days. The body temperature rises, heart and respiratory rate increases. The disease is also characterized by intermittent fever, odema, variable appetites, diarrhoea, body weakness, swelling of the eyelid, staggering, dull coats, Lachrymation and emaciation (Anosa, 1988). Also, endothelial gaps, swollen endothelial cells (Edeghere, 1980), tissue necrosis (Tizard, 1985), trypanosome-induced reproductive dysfunction (Ikede et al., 1988; Raheem, 2014), the infected animal becomes anaemic and death may result.

The plant, *Terminalia catappa* is used worldwide in the prevention and treatment of various chronic diseases (Kinoshita et al., 2007). It helps in the normalization of lipid level in liver and kidney tissues (Naitik et al., 2012) and abolishes the increase in caspase 3 activity and DNA fragmentation that were observed in the livers of treated rats (Kinoshita et al., 2007). Aqueous extract of *T. catappa* is a potential important agent for the prevention of lung cancer metastasis (Chu et al., 2007).

2. Materials and methods

2.1. Sample plant

Terminalia catappa is a large plant belonging to the family *Combretaceae.* It is also known as "INDIAN ALMOND" or "TROPICAL ALMOND" while its common name is "fruit". In Nigeria, the Northerners call it "BAUSHE"; the Westerners call it "AYIN" or **"**GBE-FON" while the Easterners call it "EDO".

2.2. Sample collection

Fresh stem barks of *Terminalia catappa* were collected in polythene bags from Mando, Kaduna, Kaduna State between the months of June and July, 2014. During sample collection, relevant plant parts that could be useful in identification were also collected and taken to the habarium unit of the Department of Biological Science Laboratory, Ahmadu Bello University, Zaria. The plant was identified by a Taxonomist using appropriate keys and assigned a voucher number (1556). Plant stem barks collected were rinsed in distilled water to remove adhering dirt and later air dried for four weeks in the laboratory until it attained a constant weight. The sample of dried stem bark was grounded to fine powder using porcelain mortar and pestle. The resultant powder was stored in brown air-tight bottles at room temperature until required (Sofowora, 1993).

2.3. Extract preparation

2.3.1. Preparation of crude ethanolic extract (CEE)

Plant extract was prepared using percolation method as described by (Garba and Okeniyi, 2012). The procedure involves weighing 500g of powdered *Terminalia catappa* and percolating in 1500cm^3 of Ethanol for 7

days in a conical flask. The resulting extract was filtered and concentrated using a rotary evaporator at 40^{o}C to obtain crude ethanol extract (CEE).

2.3.2. Phytochemical screening of extracts

Phytochemical analysis of the extract was conducted to determine the presence of secondary metabolites such as alkaloids, saponins, steroids, terpenoids and flavonoids using standard procedures as described by (Edeoga et al., 2005).

2.4. Experimental animals

15 male goats weighing between 8kg to 10kg were purchased from Afaka village market near Kaduna town in Kaduna State, Nigeria. The animals were transported immediately after purchase to Biological Science Department laboratory, Nigerian Defence Academy, Kaduna where they were screened for ecto-parasites infestation, gastro-intestinal and haemo-parasitic infection by a Veterinarian. Animals found to be infected were treated. All the animals were kept for 2 months to acclimatize during which time they were allowed free access to water and feed composed of shaft of millet, sorghum grains and dried groundnut leaves.

2.5. Housing of animals

Animals were housed in cemented floor pens that were provided with fly proof isolation unit with adequate ventilation throughout the experimental period. The pens were cleaned and disinfected with Izal daily.

2.6. Trypanosome stock

Blood sample was collected by cardiac puncture from a *T.b. brucei* infected mouse using EDTA coated syringe with needle in the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University (ABU), Zaria, Nigeria. Blood collected was immediately diluted with physiological saline to obtain $1x10^3$ parasites (trypanosomes) per microscope field. 0.1ml of infected blood containing $1x10^3$ was inoculated intraperitoneally into four uninfected mice that were to serve as donor mice. Infection was monitored every morning by microscopic examination of blood samples taken from the tails of the four infected mice until parasitaemia was established.

2.7. Experimental infection of goats / grouping

Blood sample was collected from donor mice by cardiac puncture using an EDTA coated syringe and immediately diluted with physiological saline to serve as the innoculum. Each experimental goat was infected intravenously with 2ml of the inoculum containing at least 2 to 3 parasites per field. Goats in all the groups were monitored daily for onset of parasitaemia through wet blood preparation that was examined microscopically. When parasitaemia was fully established in all groups, goats in groups 1 to 4 were treated for seven days by either oral or intraperitoneal administration of crude ethanolic extract stem bark of *T. catappa* at 50mg/kg and 100mg/kg doses respectively.

2.8. Experimental design/ grouping

Group I Crude ethanolic extract of *Terminalia catappa* administered intraperitoneally to goats (50mg/kg)
Group II Crude ethanolic extract of *Terminalia catappa* administered orally to goats (50mg/kg)
Group III Crude ethanolic extract of *Terminalia catappa* administered intraperitoneally to goats(100mg/kg)
Group IV Crude ethanolic extract of *Terminalia catappa* administered orally to goats (100mg/kg)
Group V Infected goats and untreated with crude ethanolic extract of *Terminalia catappa.*

3. Results and discussion

3.1. Phytochemical analysis of *T. catappa*

Result of phytochemical analysis of *T. catappa* crude ethanolic extract is presented in Table 1. Although the phytochemicals were not quantified, alkaloids, saponins, terpenoids and steroids were detected in *T. catappa* using crude ethanolic extraction method; however, flavonoids were not detected.

Table 1
Phytochemical constituent detected in *T. catappa* stem bark extracted using ethanolic extraction.

	Phytochemicals				
Extraction method	Alkaloids	Flavonoids	Saponins	Terpenoids	Steroids
Crude ethanolic	+	-	+	+	+

+ present, - absent

Table 2
Histopathological findings of organs of treated and untreated red Sokoto goats.

Group	Heart	Lung	Liver	Kidney	Spleen
1	NOHL	Congested	Congested central vein	Renal tubules filled with eosinophilic materials	NOHL
2	NOHL	Consolidated	NOHL	NOHL	NOHL
3	NOHL	NOHL	NOHL	Contain eosinophylic materials	NOHL
4	NOHL	Consolidated	Congested	NOHL	NOHL
5	Necrosis	Consolidation	Necrosis	Congested	Haemosiderosis

The histopathology findings carried out on selected tissues/organs of goats in treated and non-treated groups (groups 1 to 5) are shown in plates 1 to 5. Tissue sections of heart from infected and *T. catappa* treated groups showed no observable lesions (Plate1a) while heart of infected and untreated control showed necrosis (Plate 1b). Tissues sections of the lung from infected and *T. catappa* treated groups showed no observable lesions in group 3 (Plate 2a), congested lung in group 1 (Plate 2b) and consolidated lung in groups 2, 4 and 5 (control). Tissues sections of a liver showing no observable lesions in groups 2 and 3 are shown in Plate 3a, liver from infected and *T. catappa* treated groups showed congested liver in groups 1 and 4 as represented in Plate 3b, while liver of infected and untreated control showed necrosis.

Tissues sections of kidney from infected and *T. catappa* treated groups showed presence of materials in groups 1 and 3 (Plate 4b), groups 2 and 4 showed no observable lesions (Plate 4a) while kidney of infected and untreated control showed congestion. Finally, tissue sections of spleen from infected and *T. catappa* treated groups showed no observable lesions in all groups as represented in Plate 5a, while spleen of infected and untreated control showed haemosiderosis (Plate 5b).

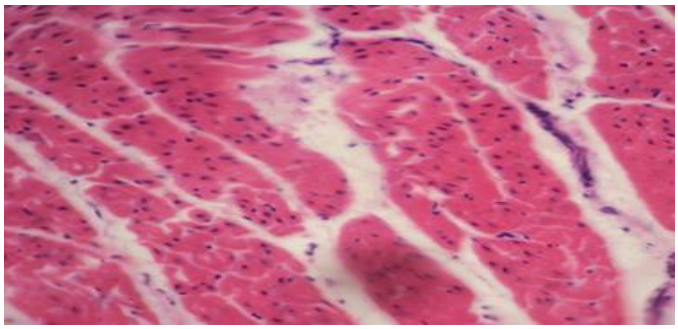

Plate 1a. Tissue section of a heart showing no observable lesion.

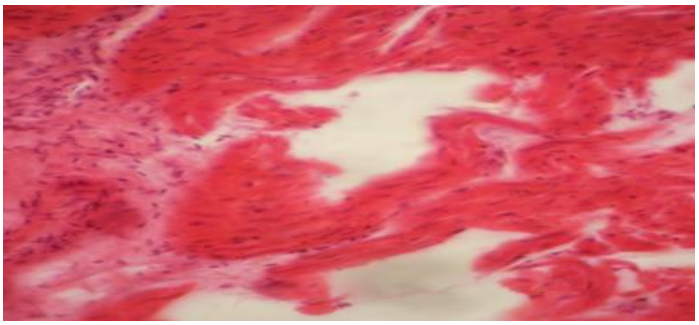

Plate 1b. Tissue section of heart from untreated group showing necrosis of the heart.

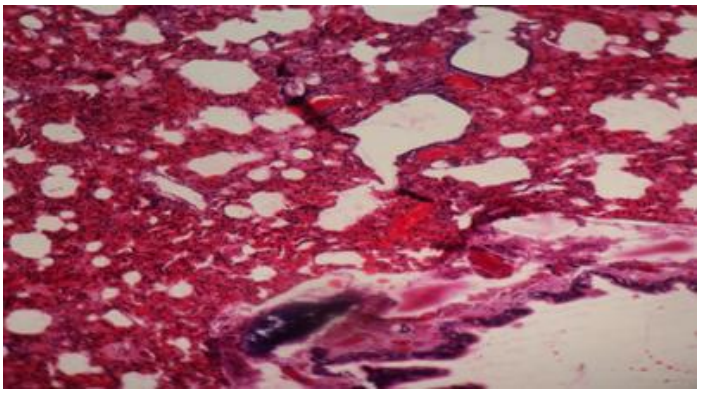

Plate 2a. Tissue section of a lung showing no observable lesion.

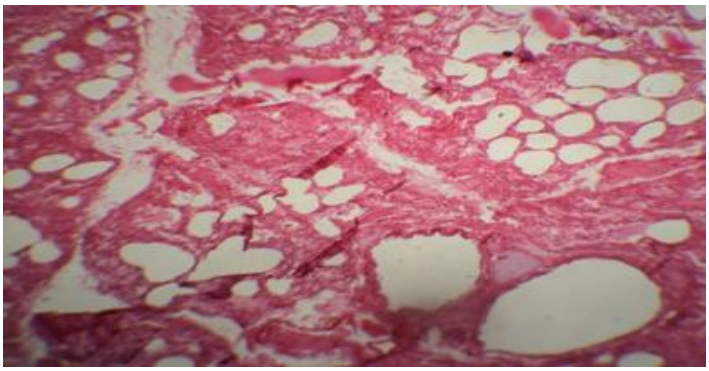

Plate 2b. Tissue section of lung from untreated group showing consolidated lung.

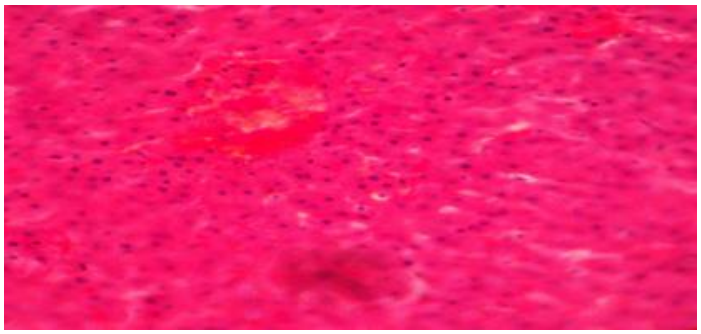

Plate 3a. Tissue section of a liver showing no observable lesion.

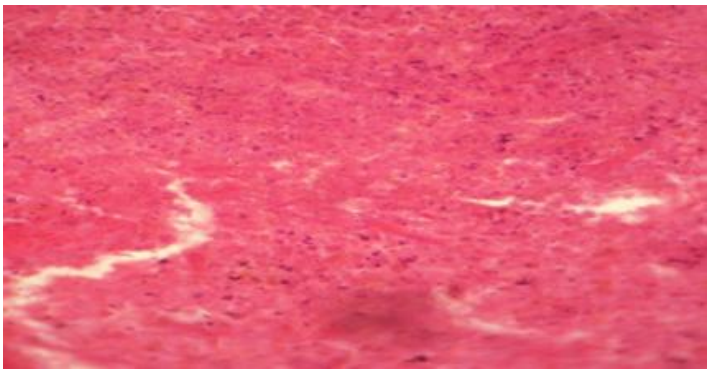

Plate 3b. Tissue section of liver from untreated group showing necrosis.

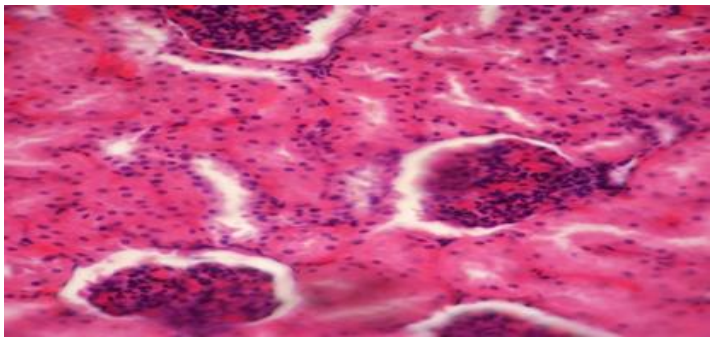

Plate 4a. Tissue section of a kidney showing no observable lesion.

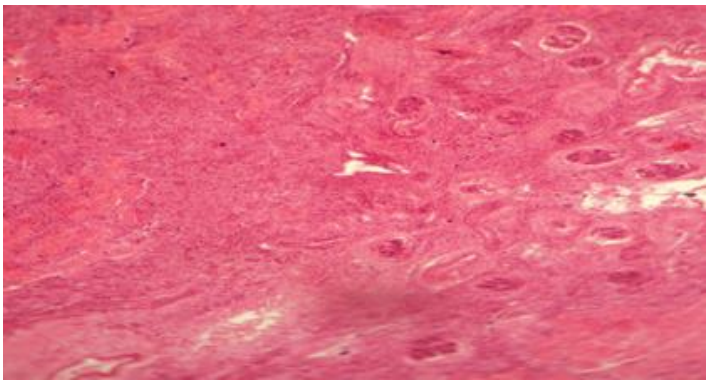

Plate 4b. Tissue section of kidney from untreated group showing congested Kidney.

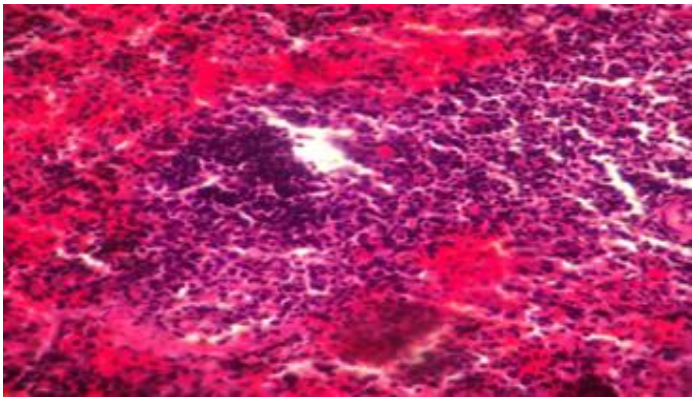

Plate 5a. Tissue section of a spleen showing no observable lesion.

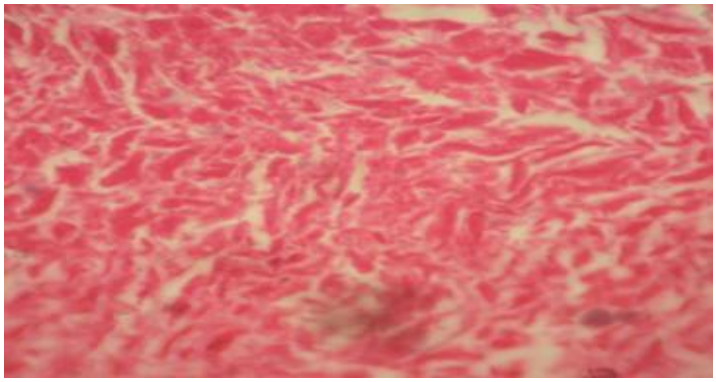

Plate 5b. Tissue section of spleen from untreated group showing Haemosiderosis.

Diseases have been reported to be a major constraint to ruminant production in Africa and Nigeria in particular as they provide up to 30% of the meat supply (Admittance et al., 2000). In this study, all goats with T*ypanosoma brucei brucei* developed parasitaemia by the 12th day after inoculation. The developed clinical manifestation in infected goats in all the groups is similar with clinical manifestations of trypanosome infected animals. The pathogenic effects of trypanosomiasis result in an acute onset of anaemia corresponding closely with the detection of parasites in the blood stream. In susceptible livestock growth, fertility and immune responses are all impaired during trypanosome infection. These important functions are influenced by hormones, some of which are small proteins produced by glands of the endocrine system. Previous studies showed that *T. b. brucei* parasite perhaps when they are dying releases enzymes into the blood stream of infected mice, which were capable of degrading host proteins (Jerry, 2014).

The trypanocidal activity of *T. catappa* extracts recorded in the present study may be due to the action of one or more constituents present in the plant. Crude ethanolic extract of *T. catappa* at dose of 50mgkg^{-1} and 100mgkg^{-1} BW was found to clear *T. b. brucei* in treated goats by the 4th day of treatment using the intraperitoneal route. The efficiency of intraperitoneal route of drug administration over the oral route using the same dosage (50mgkg^{-1} and 100mgkg^{-1}) is to be expected since oral drug administration has several drawbacks which include variable absorption of drugs, poor bioavailability due to first pass metabolism and variable drug concentration in the body which could lead to therapeutic failures.

The effectiveness of ethanolic extract could be associated with the solubility and availability of the phytochemical processes. The histopathological result showed that the hearts of all infected and treated groups showed no observable lesion while necrosis of myocardium, mononuclear cellular infiltration were observed in the heart of the infected and untreated goats (control group). Several reports have been made by (Ormerod and Venkatesan, 1970). In addition, there were gross pathology changes of myocarditis and pericardial effusion in advanced stages accompanied by dilation of the heart. (Poltera, 1985) and (Cohen, 1973) had also reported that the heart is generally considered to be the most likely organ at risk of Rhodesian sleeping sickness with death arising from pancarditis.

Consolidated and congested lungs were observed in groups 1, 2 and 3. Consolidation in lungs is one of the changes in lungs (such as bronchopneumonia in man) which is as a result of presence of infection. This has been reported by (Poltera and Cox, 1977) and (Wellde et al., 1989). Also the enlargement and hemosiderin-laden macrophages seen in the spleen of the goats had earlier been reported in infected cattle by (Anosa and Kaneko, 1983). Tubular granulomatous inflammation leading to necrosis was also observed which is similar to the observation of (Ayuba et al., 2011), (Obi et al., 2013) and (Ajuwape et al., 2006). There was congestion of the lung which concurs to the findings of (Bulus et al., 2011), who reported oedemomatous lung and severe congestion in calves.

Congested livers were observed in groups 1, 3 and 5. (Bulus et al., 2007) had reported that liver congestion appeared to be the major gross pathology accompanying treatment of infected rats with aqueous extract of *T.catappa*. Necrosis of hepatocytes, mononuclear cellular infiltration were observed in the liver of group 5. Renal tubules filled with eosinophylic materials, necrosis and congestion were observed. Liver congestion could be attributed to the presence of saponin which is known as the toxic potential of *Terminalia plant* (Igweh and Onabanjo, 1989). Saponins also have deleterious haemolysing effect on circulating erythrocytes (Igweh and Onabanjo, 1989; Sofowora, 1993). Liver congestion can also be attributed to its role in biotransformation in xenobiotics. Haemosiderosis of the spleen of two groups (WHO, 2012; Mare, 2008) were observed. This is in agreement with the works of (Anosa and Kaneko, 1983) who also observed the enlargement of the liver and spleen in patients infected with *T. rhodesiense* disease.

Acknowledgements

The authors wish to thank Dr. M. S. Abdulsallami, Dr. Vantsawa and the laboratory technologists all of the department of Biological Science and Dr. S. Garba of the department of Chemistry, Nigerian Defence Academy, Kaduna, for their support during the course of this work. Also, we wish to appreciate Dr. Okubanjo of the Department of Veterinary Parasitology and Dr. Bissala of the Department of Veterinary Pathology, both from Ahmadu Bello University, Zaria for their technical support.

References

Ajuwape, A.T.P., Adehan, R.K., Adetosoye, A.I., Ikheloa, J.O., Alaka, O.O., Ojo, M.O., 2006. Pathogenecity of Mycoplasma mycoides subsp. Capri in calves previously infected with Trypanosoma congolense. Vet. Arh., 76, 443-452.

Anosa, V.O., 1988. Haematological and biochemical changes in human and animal trypanosomiasis. Part 1. Revenue d' Elevage et de Medecine Veterinaire des pays Tropiceux. 41(1), 65-78.

Anosa, V.O., Kaneko, J.J., 1983. Pathogenesis of Trypanosoma brucei infection in deer mice (Peromyscus maniculata): Haematologic, erythrocyte biochemical, and iron metabolic aspects. Am. J. Vet. Res., 44, 639-644.

Ayuba, M., Shah, S.A., Irfan, M., Khan, J.A., Hasmi, S.O., 2011. A case of human African Trypanosomiasis during united nations mission in Liberia. Pak. Arm. Forc. Med. J., 1, 0030-9648.

Bulus, T., Atawodi, S.E., Mamman, M., 2007. Acute toxicity evaluation of Aqueous extracts of Terminalia mollis on rats. Chemclass. J., 4, 57-60.

Bulus, T., Atawodi, S.E., Mamman, M., 2011. Acute toxicity effect of the Aqueous extract of Terminalia avincennioides on white Albino rats. Sci. World. J., 6(2), 1-4.

Chu, S.C., Yang, S.F., Liu, S.J., Kuo, W.H., Chang, Y.Z., Hsieh, Y.S., 2007. In vitro and in vivo antimetastatic effects of Terminalia catappa L. leaves on lung cancer cells. Food. Chem. Toxicol., 45(7), 1194-1201.

Cohen, C., 1973. Trypanosomiasis on the witwatersrand. South Afr. Med. J., 48, 485-491.

Denbarga, Y., Ando, O., Abebe, R., 2012. Trypanossoma species causing bovine trypanosomosis in South Achefer district, Northern Ethiopia. J. Vet. Adv., (2), 108–113.

Edeghere, H.I.U.E., 1980. Morphological and ultra-structural changes in small blood vessels of rabbits infected with T. brucei. Ph.D. thesis, University of London, London.

Edeoga, H.O., Okwu, D.E., Mbaebie, B.O., 2005. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4, 685-688.

Garba, S., Okeniyi, S.O., 2012. Antimicrobial activities of total alkaloids extracted from some Nigerian medicinal plants. J. Microbiol. Antimicrob., 4(3), 60-63.

Igweh, A.C., Onabanjo, A.O., 1989. Chemotherapeutic effects of Annona senegalensis on Trypanosoma brucei brucei. Ann. Trop. Med. Parasitol., 83, 527-534.

Ikede, B.O., Elhassan, E., Akpavie, S.O., 1988. Reproductive disorders in African trypanosomiasis: A review. Acta. Trop., 34, 53-60.

Jerry, N.A., 2014. A comparative pathology of Trypanosoma brucei infection. Glob. Adv. Res. J. Med. Med. Sci. (GARJMMS), 3(12), 390-399.

Kinoshita, Y., Inoue, S., Nakama, I.T., Aniya, Y., 2007. Antioxidant and hepatoprotective actions of medicinal herb, Terminalia catappa L. from Okinawa Island and its tannin corilagin. Phytomedicine; 14, 755-762.

Malafaia, G., Talvani, A.J., 2011. Nutritional status driving infection by Trypanosoma cruzi, lessons from experimental animals. J. Trop. Med., Idoi: 10.//55/2011/981879.

Mare, C.J., 2008. African Animal Trypanosomiasis. Hhtp://www.vet.uga.edu/VPP/gray_book02/fad. Accessed 15/07/2009.

Naitik, P., Prakash, T., Kotresha, D., Rao, N.R., 2012. Effect of Terminalia catappa on lipid profile in transplanted fibrosarcoma in rats. India. J. Pharmacol., 44(3), 390-392.

Obi, C.F., Obidike, R.I., Eze, I.O., Omoja, V.U., Iheagwan, C.N., Idika, I.A., Ezeokonkwo, R.C., 2013. Effect of Trypanosoma brucei infection and diminiazine aceturate on testicular morphology and function of Nigerian local dogs. Vet. Parasitol., http://dx.doi.org/10.1016/j.vetpar.2013.03.023

Omotainse, S.O., Edeghere, H., Omoogum, G.A., Elhassan, E.O., Thompson, G., Igweh, C., Ukah, J.A.C., Ikenga, M.A., Halid, I., 2000. The prevalence of animal Trypanosomiasis in Konshisha local government area of Benue state, Nigeria. Isr. J. Vet. Sci., 55(4), 142-144.

Ormerod, W.E., Venkatesan, S., 1970. An amastigote phase of the sleeping sickness trypanosome. Transactions of Royal Society of Tropical Medicine and Hygiene. 65, 73.

Poltera, A.A., 1985. Pathology of human African trypanosomiasis with reference experimental African trypanosomiasis and infections of the central nervous system. Br. Med. Bulletin, 41, 169-174.

Poltera, A.A., Cox, J.N., 1977. Pancarditis with Valvulitis in endemoyocardial fibrosis (EMF) and in human African trypanosomiasis. A comparative histological study of four Uganda cases. Virchows, Archiv. A., 375(1), 53-70.

Raheem, K.A., 2014. A review of trypanosomiasis-induced reproductive dysfunction in male animals. Agrosearch. 14, 30-38.

Rocha, G., Martins, A., Gama, G., Brandao, F., Atouguia, J., 2004. Possible cases of sexual and congenital transmission of sleeping sickness. The Lancet; 363(9404), 247. Doi: 10,1016/S0140-673(03)15345-7. PMID 14738812.

Sofowora, A., 1993. Medicinal plants and traditional medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria. 1-10.

Tizard, I., 1985. Immunology and pathogenesis of trypanosomiasis. CCr Press. Inc. Florida.

Wellde, B.T., Reardon, M.J., Kovatch, R.M., Chumo, D.A., Williams, J.S., Boyce, W.L., Hochmeyer, W.T., Wykoff, D. E., 1989. Presenting features of Rhodesian sleeping sickness patients in the Lambwe Valley, Kenya. Ann. Trop. Med. Parasitol., 83(1), 73-90.

World Health Organisation, 2012. Human African Trypanosomiasis (sleeping sickness). Fact sheet No. 259. http://www.int/mediacentre/factsheete/fs259/en/index.html

World Health Organization (WHO), 1998. Control and surveillance of African trypanosomiasis. WHO Technical Report Series No. 881.

How to cite this article: Agbanu, J.I., Emere, M.C., Umar, Y.A., 2016. Histopathology of *Trypanosoma brucei brucei* infected red Sokoto goats experimentally infected and treated with crude ethanolic extract of *Terminalia catappa*. Scientific Journal of Veterinary Advance, 5(7), 105-113.