Manila Journal of Science ***13*** *(2020), pp. 98-108*

Effect of Tocopherol on *Trypanosoma brucei brucei*–Induced Hepatomegaly and Histopathological Indices of Male Wistar *Albino Rats*

Edoga, C.O.,[1]* Eyo, J.E.,[2] Onah, I.E.,[2] Anukwuorji, C.A.,[3] Wopara, I.,[4] and Ossai, N.I.[2]

[1]Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Nigeria
[2]Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria
[3]Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
[4]Department of Medical Biochemistry, PAMO University of Medical Sciences, Port Harcourt, Nigeria

*****E-mail: onyekachi.edoga@esut.edu.ng

ABSTRACT

The research was undertaken to determine the effect of tocopherol on *Trypanosoma brucei brucei*–induced hepatomegaly and histopathological indices of male Wistar albino rats. Fifty-four (54) male Wistar albino rats were randomly divided into six (6) groups of three (3) rats each replicated three (3) times. The rats were marked and kept in stainless wire cages labeled A–F. Groups A, B, and C were normal, negative, and standard controls, respectively. Groups D, E, and F were infected with 1.0×10^6counts per milliliter and treated with 0.5mg/kg (low dose), 2.5mg/kg (medium dose), and 5.0mg/kg (high dose) of vitamin Eper body weight per day, respectively. The experiment lasted for 21 days from the day *T. b. brucei* infection was established. The sample of serum was collected every seven days across the groups and subjected to liver function analyses. On the last day of the experiment, a sample of the liver of the experimental rats from each group was harvested and subjected to histological studies. The effects of different doses of vitamin Eon the level of biomarkers of hepatomegaly between the treated groups were compared against the controls. The serum levels of aspartate

aminotransferase (AST) (U/L), alkaline phosphatase (ALP) (U/L), and alanine aminotransferase (ALT) (U/L) were determined. There wasa significant difference ($p < 0.05$) in the effect of tocopherol on the concentrations of the indicators of hepatomegaly and the duration of the experiment. These reductions were seen in AST, ALP, and ALT between the treated groups and the normal control. After treatment, AST, ALP, and ALT levels differed significantly ($p < 0.05$) between the untreated, treated, and control groups. In conclusion, the intervention ameliorated the trypanosome-induced hepatomegaly observed in the parameters measured in the male Wistar albino rats. There was observable infiltration of the periportal areas to degeneration and necrosis on the liver tissues of the negative control group when compared to those of the vitamin E–treated group that showed mild inflammatory changes.

Keywords: *Trypanosoma brucei brucei,* tocopherol, hepatomegaly, AST, ALT, ALP

INTRODUCTION

Trypanosomes are protozoan hemoflagellates of the genus *Trypanosoma.* There are many species of trypanosomes, but only two subspecies of *T. brucei* are the cause of sleeping sickness in man. The subspecies can be identified by the use of a microscope but are morphologically similar (World Health Organization, 2013). Trypanosome is transmitted by the tsetse fly (*Glossina* spp.). They are characterized by a complex lifecycle (Rotureau, Van, & Abbeele, 2013), with differential biological stages in both the insect vector and the mammalian host. When entering into the tsetse fly, the trypanosomes are ingested as bloodstream trypomastigotes and proceed to the midgut. Some of the trypomastigotes in the insect's midgut may differentiate into procyclic forms, which replicate *insitu* and pass the peritrophic membrane to reach the proventriculus to become monocyclic trypomastigotes and later epimastigotes (Dyer, Rose, Ejeh, & Acosta-Serrano, 2013). Humans constitute the main epidemiological reservoir of *T. b. gambiense* (Franco, Simarro, Diarra, Ruiz-Postigo, & Jannin, 2014). Symptoms of human trypanosomiasis are dependent on the stage of the trypanosomes within the host. In the hemolymphatic stage, the patient may exhibit the following symptoms: fever, skin disorders, headache, malaise, exhaustion, irregular febrile episodes, anorexia, extreme thirst, muscle and joint pains, anemia, rashes, pruritus, deep hyperesthesia, feeling of coldness, lack of appetite, hyperphagia, polydipsia, impotence, amenorrhea, organ dysfunctions, daytime somnolence, and nighttime insomnia (Vincendeau & Bouteille, 2006). In meningoencephalitis, the following symptoms appear: headaches, sensory disturbance, primitive reflexes, exaggerated deep tendon reflexes, psychiatric disorders, tremor, disruption of body temperature regulation, disruption of cortisol, prolactin or growth hormone secretions, central nervous system demyelination and atrophy, dementia, epileptic fits, and ultimately death (Vincendeau & Bouteille, 2006). Hepatomegaly is a disease of the liver characterized by enlargement of the liver tissues. It is clinically diagnosed by deviations in the levels of the primary liver

biomarkers (Anderson, 2002). The liver function enzymes have been implicated as the essential biomarkers of hepatomegaly. Elevated serum enzymes have been reported in monkeys suffering from trypanosomiasis (Nguru *et al.,* 2008). Yusuf, Umar, and Nok (2012) found that trypanosome-induced liver enzymes can lead to liver damage caused by proliferating parasites. There are available trypanocidal drugs for trypanosomiasis; however, the drugs are scarce, lethal, unaffordable, and highly resistant (Reyes & Vallejo, 2005). These challenges informed the choice of tocopherol. Vitamin E is found naturally in some foods, added to others, and available as a dietary supplement. It is a fat-soluble compound with antioxidant functions (Traber, 2006). Tocopherol occurs in various chemical forms: alpha-, beta-, gamma-, and delta-tocopherol, which have varying levels of biological functions (Traber, 2006). Alpha-tocopherol is the only form that can resecrete in the liver via the hepatic alpha-tocopherol transfer protein (Traber, 2006). Several studies have been documented on the antitrypanosomal effects of vitamin E. The work of Yakubu, Dawet, and Olaleye (2014) found that vitamin E caused a significant reduction in parasitemia levels of *T. b. brucei*–infected rats. Dietary supplementation of tocopherol can enhance the resistance of trypanosomiasis (Ufele, Mgbenka, & Ude, 2007).The broad objective of the study was to determine the effect of tocopherol on *Trypanosoma brucei brucei*–induced hepatomegaly and histopathological indices of male Wistar albino rats. The specific objectives were to1)determine the effect of tocopherol on alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) of albino rats infected with *T. b. brucei*;2)determine the effect of tocopherol on histological indices of liver tissues; and3)determine the damaging effect of trypanosomiasis on liver tissues.

MATERIALS AND METHOD

Animal Model and Experimental Protocol

Fifty-four (54) male albino Wistar rats (*Rattus norvegicus*) aged 3 months, weighing between 180 and 220g, were procured, housed, and allowed to acclimatize for two weeks at the Pharmacy Animal House, Madonna University, Elele, Rivers State. The research was approved by the Ethics Board of the Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria. The rats were grouped into six (6) cages labeled A–F comprising three (3) rats that were replicated three (3) times from each group. The animals were kept under normal room temperature with ad libitum access to feed and water. The cages were cleaned daily to prevent infection of the animals and to minimize extraneous variables. The groups (A–F) were as follows: Group A (normal control) was neither infected with trypanosomes nor treated with vitamins; Group B (negative control) was infected with 1.0×10^6counts per milliliter but not treated; Group C (standard control) was infected with 1.0×10^6counts per milliliter and treated with 0.2 mg/kg body weight of diminazeneaceturate; Group D (low dose) was infected with 1.0×10^6counts per milliliter and treated with 0.5 mg/kg body weight of vitamin E per day; Group E (medium dose) was infected with 1.0×10^6counts per milliliter and treated with 2.5 mg/kg body weight of vitamin E per day; Group F (high dose) was infected with 1.0×10^6counts per milliliter and treated with 5.0 mg/kg body weight of vitamin E. The experiment lasted for 21 days after *T. b. brucei* infection was established. A sample of serum was collected weekly from the three (3) rats across the groups and taken to Divine Chemicals and Analytical Laboratory, Nsukka, for liver function analyses. On the last day of the experiment, one liver tissue from each group was

harvested and taken to the Anatomical Pathology Unit, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, for histopathological studies.

Procurement and Inoculation of Trypanosomes

T. b. brucei was obtained from an experimentally infected rat previously inoculated with the parasite from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Each experimental rat was administered 0.1mLof infected blood in 0.3mL of normal saline containing 1×10^6counts per milliliter using the rapid matching method (Herbert & Lumsden, 1976) to determine the level of parasitemia. Inoculation was done intraperitoneally.

Determination of Parasitemia

Wet blood preparations were covered with a coverslip on a slide and examined under ×40 and oil immersion using a microscope at ×100 magnification. The identification of parasites was done using morphological description (Van-Wyk & Mayhew, 2013).

Formulation and Administration of Vitamin E

Vitamin E (alpha-tocopherol) was procured at Science Line, New Parts, Onitsha, Anambra State, Nigeria, in a powder bottle. The working concentrations were weighed at the Departmental of Biochemistry, Madonna University, Elele, from the result of acute oral toxicity (LD_{50}) test of vitamin B_{12} as follows:

Mild dose:0.5 mg/kg body weight per day
Enriched dose: 2.5 mg/kg body weight per day

High dose:5.0 mg/kg body weight per day

The working concentrations were dissolved in 2% ethanol as a vehicle and administered via intubation.

Standard Drug

Diminazeneaceturate was procured from the Faculty of Veterinary Medicine Clinic, University of Nigeria, Nsukka, Nigeria, in 2.36-g granules. The working dosage was 0.2mg/kg. The administration was intravenous.

Clinical Determination of Liver Function

Blood samples for clinical determination of liver and renal functions were collected from the retrobulbar plexus of the medial canthus of the eye of the rats. The blood sample was kept at room temperature for 30 min to clot. Afterward, the test tube containing the clotted blood sample was centrifuged at 3,000 revolutions per minute for 10 min using a table centrifuge to enable complete separation of the serum from the clotted blood. The clear serum supernatant was carefully collected with a syringe and needle and stored in a clean sample bottle for the liver function determinations.

AST/ALT. The estimation of serum activities of AST and ALT was carried out according to the methods of Reitman and Frankel (1957) using Randox Test Kits.

ALP. The plasma ALP activity was determined using Randox Test Kits (Randox Laboratories Ltd., USA) according to Kind and King (1954).

Histopathological Examination

Tissue preparation. The surviving experimental animals were humanely

sacrificed at the end of the study. Gross lesions were recorded as observed during the postmortem examination. Sections of the heart, liver, and kidneys were collected, prepared, and examined for histopathological changes.

The samples after excision were fixed in 10% phosphate-buffered formalin for 72 hr. The tissues were subsequently trimmed, dehydrated in fourgrades of alcohol (70%, 80%, 90%, and absolute alcohol), cleared in threegrades of xylene, and embedded in molten wax. On solidifying, the blocks were cut into 5-µm-thick tissue sections using a rotary microtome, floated in a water bath, and incubated at 60°C for 30 min. The 5-µm-thick sectioned tissues were subsequently cleared in threegrades of xylene and rehydrated in threegrades of alcohol (90%, 80%, and 70%). The sections were then stained with hematoxylin for 15 min. Blueing was done with ammonium chloride. Differentiation was done with 1% acid alcohol before counterstaining with eosin. Permanent mounts were made on degreased glass slides using a Dibutylphthalate Polystyrene Xylene (DPX) mountant.

Slide examination and photomicrography. The prepared slides were examined with a Motic™ compound light microscope using ×4, ×10, and ×40 objective lenses. The photomicrographs were taken using a Motic™ 2.0-megapixel microscope camera at ×160 and ×400 magnifications.

Statistical Analysis

The values of the parameters were expressed as mean ± SEM. Data were subjected to a two-way analysis of variance using the SPSS software for Windows (version 21), and the difference between means was separated usingDuncan's multiple range tests. The test for significance was considered at the 0.05 probability level.

RESULTS

Biochemical Parameters

AST concentration. The results of mean AST level (U/L) at short and long periodswere compared in various groups. A similar pattern occurred at various points during the experiment. On the 14thday after infection (Week 2), the result indicated a significant increase in the negative control (64.333 ± 3.283) when compared to the normal control (32.000 ± 3.055) ($p < 0.05$) (Table 1). There were no significant differences in the mean serum AST between the normal and standard controls at Weeks 1, 2, and 3 after infection ($p > 0.05$).

There was a significant difference in the negative control in mean serum AST concentration withinthe duration of the study ($p < 0.05$). This showed that the mean serum AST level increased as the parasitemia increased. A comparison of mean serum AST levels in vitamin E–treated groups showed there was a significant reduction in the level of AST when compared to the negative control at Weeks 1, 2, and 3 after infection and treatment.

Table 1. Effect of Tocopherol on Serum Aspartate Aminotransferase (AST) Level (U/L)

Groups	Week 1	Week 2	Week 3
A	$32.667 \pm 3.842^{a,1}$	$32.000 \pm 3.055^{a,1}$	$33.000 \pm 3.786^{a,1}$
B	$59.000 \pm 0.577^{b,1}$	$64.333 \pm 3.283^{b,2}$	$64.000 \pm 5.508^{b,2}$
C	$36.667 \pm 0.882^{a,1}$	$34.667 \pm 0.882^{a,1}$	$32.333 \pm 0.882^{a,2}$
D	$40.667 \pm 1.202^{c,1}$	$38.667 \pm 1.856^{c,1}$	$42.667 \pm 1.202^{c,1}$
E	$43.333 \pm 3.383^{c,1}$	$40.667 \pm 1.202^{c,d,1}$	$43.667 \pm 0.082^{c,1}$
F	$38.667 \pm 6.489^{a,1}$	$43.333 \pm 3.383^{d,1}$	$40.333 \pm 2.082^{c,1}$

Note. Mean values (mean ± SEM) with the same superscriptletters and numbers per column and per row, respectively, denote no significant differences ($p > 0.05$).

ALT concentration. The results of mean ALT level (U/L) at short and long periodswere compared in various groups. A similar pattern occurred at various points duringof the experiment. On the third week after infection, the result showed a significant increase in the negative control (92.000 ± 2.517) when compared to the normal control (42.000 ± 0.577) ($p < 0.05$) (Table 2). There were no significant differences in the mean serum ALT between the normal and standard controls at Weeks 1, 2, and 3 after infection ($p > 0.05$).

There was a significant difference in the negative control in mean ALT concentration withinthe duration of the study ($p < 0.05$). This showed that the mean cardiac ALT level increased with increased infectivity. A comparison of mean ALT levels in vitamin E–treated groups showed there was a significant reduction when compared to the negative control at Weeks 1, 2, and 3 after infection and treatments.

Table 2. Effect of Tocopherol on Serum Alanine Aminotransferase (ALT) Level (U/L)

Groups	Week 1	Week 2	Week 3
A	$41.667 \pm 0.882^{a,1}$	$40.667 \pm 0.882^{a,1}$	42.000 ± 0.577^{a1}
B	$63.667 \pm 2.848^{b,1}$	$80.333 \pm 5.898^{b,1}$	$92.000 \pm 2.517^{b,2}$
C	$44.667 \pm 2.729^{a,1}$	$43.667 \pm 0.667^{a,1}$	$45.000 \pm 0.577^{a,1}$
D	$54.667 \pm 0.333^{c,1}$	$50.000 \pm 0.577^{c,1}$	$56.000 \pm 1.155^{c,2}$
E	$50.000 \pm 0.577^{c,1}$	$51.667 \pm 1.453^{c,1}$	$55.000 \pm 2.082^{c,2}$
F	$46.333 \pm 0.882^{a,1}$	$44.333 \pm 1.453^{a,1}$	$52.333 \pm 2.082^{c,2}$

Note. Mean values (mean ± SEM) with the same superscript letters and numbers per column and per row, respectively, denote no significant differences ($p > 0.05$).

ALP concentration. The results of mean ALP level (U/L) at short and long periodswere compared in various groups. A similar pattern occurred at various pointsduringthe experiment. On the seventh day after infection, the result showed a significant increase in the negative control (22.857 ± 0.107) when compared to the normal control (19.341 ± 0.422) ($p < 0.05$) (Table 3). There were no significant differences in the mean serum ALP between the normal and standard controls at Weeks 1, 2, and 3 after infection ($p > 0.05$).

There was a significant difference in the negative control in mean ALP concentration within the duration of the study ($p < 0.05$). This showed that the mean cardiac ALP level increased as infectivity increased. A comparison of mean ALP levels in vitamin E–treated groups showed there was a significant reductions when compared to the negative control at Weeks 1, 2, and 3 after infection and treatments.

Table 3. Effect of Tocopherol on Serum Alkaline Phosphatase (ALP) Level (U/L).

Groups	Week 1	Week 2	Week 3
A	$19.341 \pm 0.422^{a,1}$	$19.690 \pm 0.576^{a,1}$	$19.980 \pm 0.193^{a,1}$
B	$22.857 \pm 0.107^{b,1}$	$23.562 \pm 0.154^{b,2}$	$23.035 \pm 0.259^{b,3}$
C	$20.071 \pm 0.281^{a,1}$	$19.980 \pm 0.176^{a,1}$	$20.255 \pm 0.527^{a,1}$
D	$20.611 \pm 0.500^{c,1}$	$21.529 \pm 0.712^{c,1}$	$21.504 \pm 0.160^{c,1}$
E	$20.306 \pm 0.105^{c,1}$	$20.685 \pm 0.298^{c,1}$	$21.274 \pm 0.039^{c,1}$
F	$20.653 \pm 0.033^{c,1}$	$20.913 \pm 0.280^{c,1}$	$20.980 \pm 0.415^{a,1}$

Note. Mean values (mean ± SEM) with the same superscriptletters and numbers per column and per row, respectively, denote no significant differences ($p > 0.05$).

Histopathology of Liver Tissues

Normal control. Sections of the liver showed normal hepatic lobules with normal hepatocytes arranged in interconnecting, radiating cords around the central veins. The hepatic cords radiate towards the periphery of the hepatic lobules where they meet with the portal area, which contains the portal triads (Plate 1).

Negative control. Sections of the liver collected from this group showed mild to moderate infiltration of the periportal areas by inflammatory mononuclear cells (Plate 2).

Standard control. Sections of the liver collected from this group showed very mild inflammatory changes (Plate 3).

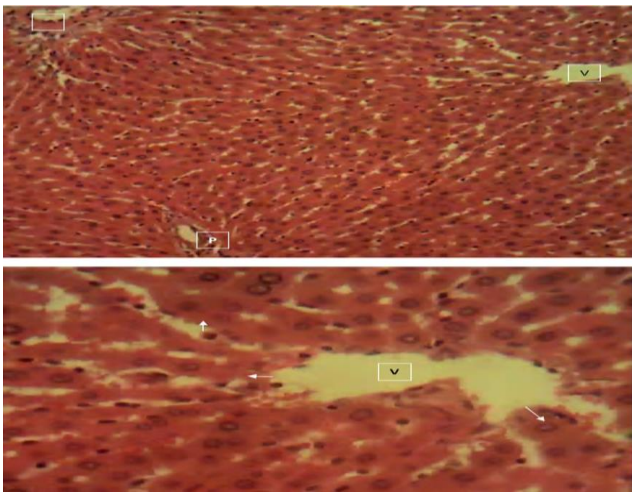

Plate 1. Transverse section of the liver of the rat in Group A.V= central vein; P=portal area; arrow=hepatic cord (H & E, ×160 and ×400).

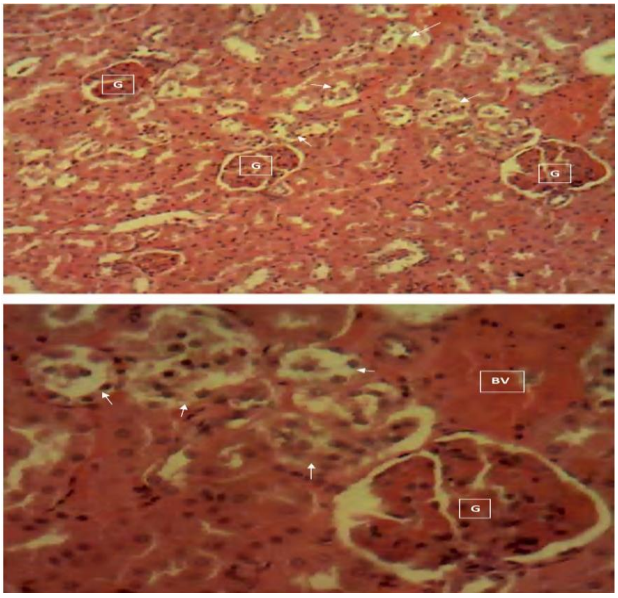

Plate 2. Transverse section of the liver of the rat in Group B (H & E,×160 and ×400).

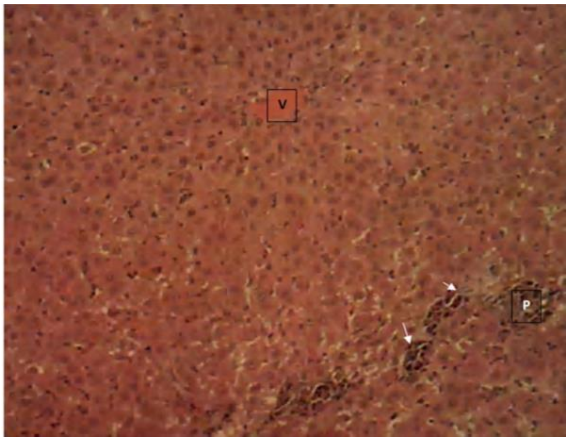

Plate 3. Transverse section of the liver of the rat in Group C.V = central vein; P= portal area; arrow = inflammatory cells (H & E,×160).

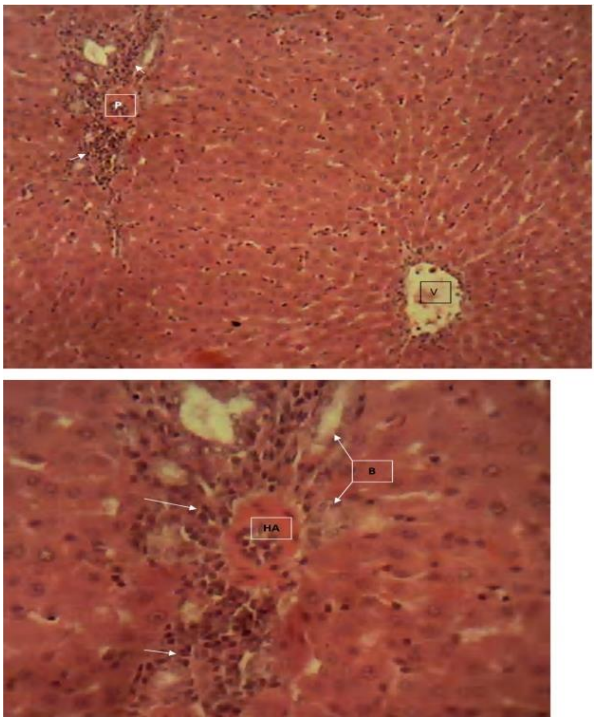

Plate 4. Transverse section of the liver of the rat in Group D. V = central vein; P = portal area; B = bile duct; HA = hepatic artery; arrow = inflammatory cells (H & E, ×160 and ×400).

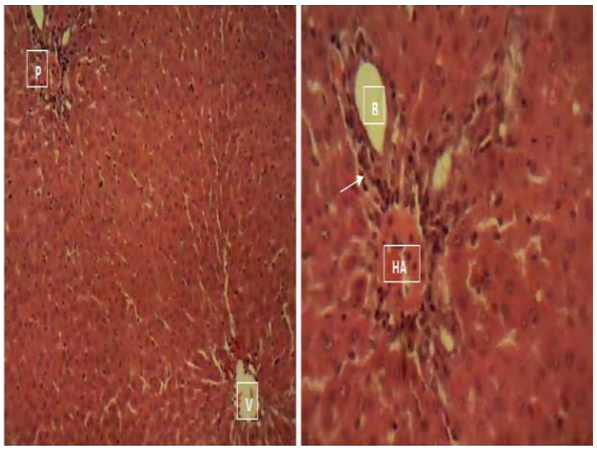

Plate 5. Transverse section of the liver of the rat in Group E. V = central vein; P = portal area; B = bile duct; HA = hepatic artery; arrow = inflammatory cells (H & E,×160 and×400).

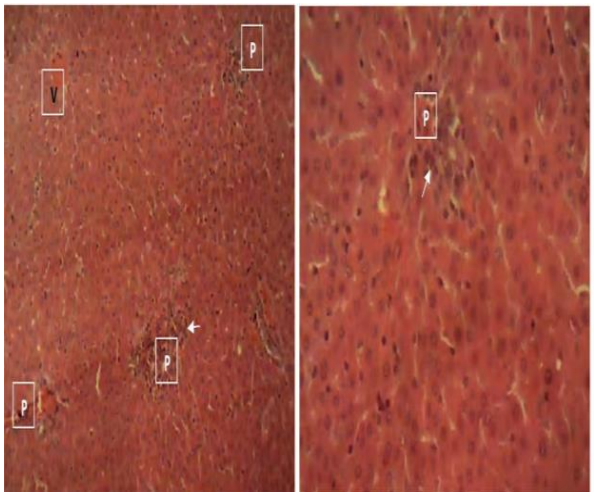

Plate 6. Transverse section of the liver of the rat in Group F.V = central vein; P = portal area; arrow = inflammatory cells (H & E,×160 and×400).

Low dose (Group D). Sections of the liver collected from this group showed very mild inflammatory changes (Plate 4).

Medium dose (Group E). Sections of the liver collected from this group showed very mild inflammatory changes. There was mild infiltration of inflammatory leucocytes around the portal areas (Plate 5).

High dose (Group F). Sections of the liver collected from this group showed very mild inflammatory changes. There was mild infiltration of inflammatory leucocytes around the portal areas (Plate 6).

DISCUSSION AND CONCLUSION

Discussion

The significant high levels of liver enzyme parameters AST, ALT, and ALP with a surge in trypanosomal infection shown in this present study suggest that the degree of liver cell necrosis that is usually present in cirrhosis may be the cause of the increase in these parameters. The increase of these liver function enzymes agrees with the work of Umaret *al.(*2001), who reported elevated liver enzymes in trypanosome-infected rats. The present study aligns with the observations of Sazmand, Aria, Mohammad, Hosein, and Seyedhossein (2011), who reported a significant increase in liver enzyme concentrations in dromedary camels infected with *T. evansi*, and Yusuf et al.(2012), who observed elevated liver function enzymes in Wistar rats with alloxan-induced hyperglycemia. The increase of these enzymes is usually indicative of liver damage caused by lysis or destruction by the trypanosomes (Yusuf et al., 2012).

Treatments with diminazeneaceturate and tocopherol significantly retarded the disease-induced increase in serum AST, ALT, and ALP, agreeing with the reports of Omobowale, Oyagbemi, Oyewunmi, and Adejumobi (2015), who reported the chemo preventive effect of methanolic extract of *Azadirachta indica* on experimental *T. brucei*–induced increase in liver enzyme function. This is suggestive that vitamin E protected the liver against disease-generated oxidative species during the disease process probably by its oxidative property that protected liver cells against reactive oxygen

species. There was an infiltration of the periportal areas by inflammatory mononuclear cells to degeneration and necrosis in the negative control. This is in line with the work of Darganetes, Compbell, Copeman, and Reid (2005)on goats infected with *T. evansi.* However, the groups infected and treated with vitamin E showed mild to very mild inflammatory changes. This is consistent with the observations of Harald, Kathrin, Dietmar, and Johanna (2014) and Amindeet *al.*(2017),who reported an inverse relationship between vitamin E and cardiovascular risk factors.

Conclusion

The result of the present study indicated that infection with *T. brucei brucei*ca used an evidenced increase in the activities of serum AST, ALT, and ALP. However, the administration of vitamin B_{12} caused a dose-dependent reduction in the concentrations of AST, ALT, and ALP.

REFERENCES

Aminde, L.N., Takah, N., Ngwasiri, C., Noubiap, J. J., Tindong, M., Dzudie, A., &Veerman, J. L. (2017). Population awareness of the cardiovascular disease and its risk factors in Buea, Cameroon.*Public Health, 17*, 545.

Anderson, D. M. (2002). Definition of hepatomegaly.In *Mosby's medical, nursing, and allied health dictionary* (6th UK ed.). St. Louis, Missouri, USA: Mosby.

Darganetes, A.P., Compbell, R. S. F., Copeman, D. B., & Reid, S.A. (2005). Experimental *Trypanosoma evansi* infection in the goat. *Journal of Comparative Pathology, 133*, 267–276.

Dyer, N. A., Rose, C., Ejeh, N. O.,& Acosta-Serrano, A. (2013). Flying tryps: Survival and maturation of trypanosomes in tsetse flies. *Trends in Parasitology, 29*(4), 188–196.

Franco, J.R., Simarro, P.P., Diarra, A., Ruiz-Postigo, J. A., & Jannin, J.G. (2014). The journey towards the elimination of *gambiense* human African trypanosomiasis: Not far, not easy. *Parasitology,141*(6), 748–760.

Harald, M., Kathrin, B., Dietmar, F., & Johanna, M.G. (2014). Antioxidants, inflammation and cardiovascular diseases. *World Journal of Cardiology,6*(6), 462–477.

Herbert, W. J., &Lumsden, W.H.R. (1976). *Trypanosoma brucei*: A rapid matching method for estimating the host's parasitemia. *Experimental Parasitology*, *40*, 427–431.

Kind, P. R., & King, E.J. (1954). Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-antipyrine. *Pathology, 7*(4), 322–326.

Nguru, R.M., Ndungu, J.M., Ngotho, J.M., Nancy, M. K., Maathai, R. G., & Gateri, L.M. (2008). Biochemical changes in the plasma of vervet monkeys *(Chloroce busaethiops)* experimentally infected with *Trypanosoma bruceirhodesiense. Journal of Cell and Animal Biology, 2*(7), 150–157.

Omobowale, T.O., Oyagbemi, A. D., Oyewunmi, O. A., & Adejumobi, O.A. (2015). Chemopreventive effect of methanolic extract of *Azadirachta indica* on experimental *Trypanosoma brucei* induced oxidative stress in dogs. *Pharmacognosy Research,7*(3), 249–258.

Reitman, S., & Frankel, S. (1957). A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, *28*, 56–62.

Reyes, P. A., & Vallejo, M. (2005).Trypanocidal drugs for late-stage, symptomatic Chagas disease (*Trypanosoma cruzi* infection). *Cochrane Database Systematic Reviews, 4.*

Rotureau, B., Van, D.,&Abbeele, J. (2013). Through the dark continent: African trypanosome development in the tsetse fly. *Frontiers in Cellular andInfection Microbiology*, *3*(53),1–7.

Sazmand, A., Aria, R., Mohammad, N., Hosein, H., & Seyedhossein, H. (2011). Serobiochemical alterations in subclinically affected dromedary camels with *Trypanosoma evansi*in Iran.*Pakistan Veterinary Journal, 31*(3), 223–226.

Traber, M. G. (2006).Vitamin E. In M. E. Shils, M. Shike, A. C. Ross, B. Caballero, &R. Cousins (Eds.),*Modern nutrition in health and disease*(10th ed.) (pp. 396–411). Baltimore, MD: Lippincott Williams & Wilkins.

Ufele, A. N., Mgbenka, B. O., & Ude, J.F. (2007). Effect of food supplementation on the white blood cell count and differential leucocytes count of trypanosome-infected pregnant rats. *Animal Research International, 4*(2), 643–646.

Umar, I.A., Igbalajobi, F.I., Toh, Z.A., Gidado, A., Shugaba, A., & Buratai, L.B. (2001). Effects of repeated daily doses of vitamin E (alpha-tocopherol) on some biochemical indices of rats infected with *T. brucei. West African Journal Biological Sciences, 12*, 1–7.

Van-Wyk, J. A., & Mayhew, E. (2013). Morphological identification of parasitic nematode infective larvae of small ruminants and cattle: A practical lab guide. *Journal of Veterinary Research*, *80*, 1.

Vincendeau, P.,&Bouteille, B. (2006). Immunology and immunopathology of African trypanosomiasis. *Anais da Academia Brasileira de Ciencias,78*,645–665.

World Health Organization. (2013). *The 17 neglected tropical diseases*. World Health Organization. Retrieved from http://www.who.int/neglected-diseases/diseases/en/

Yakubu, D. P., Dawet, A., &Olaleye, N.A. (2014). Effect of vitamin E on some blood parameters of *Trypanosoma brucei brucei*-infected rats. *British Journal of Applied Science and Technology,4*(7), 1100–1108.

Yusuf, A.B., Umar, I.A.,&Nok, A.J. (2012). Effect of methanol extract of *Vernonia amygdalina* leaf on survival and some biochemical parameters in acute *Trypanosoma brucei brucei* infection. *African Journal of Biochemical Research, 6*(12),150–158.