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Effect of Tocopherol on *Trypanosoma brucei brucei*-Induced Nephropathy and Histopathological Indices of Male Wistar Albino Rats

C. O. Edoga[1*], and C. A. Anukwuorji[2]

[1]Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Nigeria
[2]Department of Botany, Nnamdi Azikiwe University, 5025 Awka, Anambra State, Nigeria

*Email: onyekachi.edoga@esut.edu.ng; dozygreat@gmail.com

ABSTRACT

The research aimed at determining the effect of tocopherol on *T. b. brucei*-induced nephropathy and histopathological indices of male Wistar albino rats. Fifty-four male Wistar albino rats were 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 1.0×10^6 trypanosomes and treated with 0.5 mg (low dose), 2.5 mg (medium dose), and 5.0 mg (high dose) of vitamin E per kilogram of body weight per day, respectively. The sample of serum was collected weekly across the groups and subjected to kidney function analyses. After one month, a sample of the kidney of the rats was harvested for histological studies. The effects of different doses of vitamin E on the level of biomarkers of hepatomegaly between the treated groups were compared against the controls. There was a significant difference ($p < 0.05$) in the effects of the levels of the biomarkers of nephropathy, which were also dependent on the duration of the study. There were multifocal degenerations, congestion, necrosis, and morphologic alterations in the renal tissues of the negative control group, when compared to the vitamin E-treated groups. In conclusion, vitamin E ameliorated the trypanosome-induced nephropathy observed in the parameters measured in the *T. b. brucei*-infected rats.

Keywords: *Trypanosoma brucei brucei*, tocopherol, nephropathy, urea nitrogen, creatinine

INTRODUCTION

Human African trypanosomiasis (HAT) is one of the diverse ranges of neglected diseases that are widespread in Africa. It is a focal disease, which means that the distribution of the disease is restricted to a particular area, called a "focus," beyond which the disease does not take place (Welburn & Maudlin, 2012). However, environmental fluctuations and human or livestock can change the transmission of HAT or even geographical settings (Stone & Chinis, 2015). Though sleeping sickness was first described around the 14th century, it was considered endemic in Africa since the appearance on human beings (Rodiit & Lehane, 2008). Two types

of trypanosomiasis are known: African trypanosomiasis (sleeping sickness in humans and/or nagana in animals) and American trypanosomiasis, also known as Chagas disease. HAT is predominantly distributed across most of the 38 countries of sub-Saharan Africa that are endemic for tsetse flies and the disease (Cecchi et al., 2014). The epidemiology of HAT currently covers an area of 8 million km^2 between 14° north and 20° south latitude (Franco et al., 2014). There are two stages of African trypanosomiasis. The first stage of the disease (hemolymphatic stage) is characterized by the localization of the trypanosomes to the blood and lymphatic system (World Health Organization, 2006). The stage presents the following symptoms: fever, headache, joint pains, itching, and organ dysfunction. The second stage (meningoencephalitis) presents the following symptoms: confusion, disturbed sleep pattern, sensory disturbances, extreme lethargy, poor condition, coma, and death (World Health Organization, 2006). Trypanosomiasis in the blood produces fluctuations in biochemical parameters (Taiwo et al., 2003). Several of these changes have been reported in serum urea nitrogen and creatinine (Nguru et al., 2008). Nephropathy is a disease of the kidney that is characterized by high concentrations of urea nitrogen and creatinine (Masakazu et al., 2015). Serum urea is the major nitrogenous waste product of metabolism and generated from protein breakdown. High urea nitrogen was reported in the plasma of vervet monkeys infected with *T. b. rhodesiense* (Nguru et al., 2008). Increased serum creatinine concentration was accompanied by a corresponding increase in the parasitemia of *T. b. brucei*-infected rats (Allam et al., 2011). The high serum creatinine concentration was due to the destruction of kidney cells by *T. b. brucei* (Ezeokonkwo et al., 2012). Vitamin E is a known chain-breaking antioxidant hindering lipid peroxidation (Lesenefsky et al., 2001). It is a hydrophobic antioxidant found in lipoprotein membranes and can confer primary and secondary protection. Vitamin E is described as the most potent antioxidant and effective scavenger of lipid peroxyl radicals (Niki, 2014). Numerous studies have reported an inverse relationship between antioxidant intake and cardiovascular risk factors (Harald et al., 2014; Jane & Leopold, 2015). Vitamin E, mostly α-tocopherol, can ameliorate the modifiable indexes by modulating free radical production (Kaya, 2009). Vitamin E has been established to cause a reduction in oxidative stress status in the small intestine of diabetic rats (Shirpoor et al., 2007). Vitamin E functions also in immunity, cell signaling, regulation of gene expression, and other metabolic processes (Traber, 2006). Alpha-tocopherol hinders the function of protein kinase C, an enzyme responsible for cell proliferation and differentiation in smooth muscle cells, platelets, and monocytes (Institute of Medicine, Food and Nutrition Board, 2000). It also enhances the expression of two enzymes that prevent arachidonic acid metabolism, thereby inducing the release of prostacyclin from the endothelium, which, in turn, increases the diameter of blood vessels and hinders platelet aggregation (Institute of Medicine, Food and Nutrition Board, 2000). Various studies have demonstrated that supplemental vitamin E functions in immunity (Moriguchi & Muraga, 2000). Also, similar results had been reported on the role of dietary vitamin E supplement on the differentiation of immature T cells in the thymus. Satoru and Mayumi (2003) observed that vitamin E deficiency caused a decrease in the differentiation of immature T cells in the thymus, which results in the early reduction of cellular immunity with aging in spontaneously hypertensive rats. The role of vitamin E in the interplay between nerves and muscles remains elusive. The function of vitamin E

in human reproduction has not been established. Several observations have been documented on the antitrypanosomal effects of vitamin E. The work of Yakubu et al. (2014) reported that vitamin E caused a significant reduction in parasitemia levels of *T. b. brucei*-infected rats. Dietary supplementation of vitamin E can enhance the resistance of trypanosomiasis (Mgbenka & Ufele, 2004; Ufele et al., 2007).

MATERIALS AND METHOD

Animal Model and Experimental Protocol

Fifty-four (54) male albino Wistar rats (*Rattus norvegicus*) aged 3 months and weighing between 180 and 220 g were procured, housed, and allowed to acclimatize for two weeks at the Pharmacy Animal House, Madonna University, Elele, Rivers State. 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 thus: Group A (normal control) was neither infected with trypanosomes nor treated with vitamins; Group B (negative control) was infected with 1.0×10^6 trypanosomes but not treated; Group C (standard control) was infected with 1.0×10^6 trypanosomes and treated with 0.2 mg of diminazene aceturate per kilogram of body weight; Group D (low dose of vitamin E) was infected with 1.0×10^6 trypanosomes and treated with 0.5 mg of vitamin E per kilogram of body weight; Group E (medium dose of vitamin E) was infected with 1.0×10^6 trypanosomes and treated with 2.5 mg of vitamin E per kilogram of body weight; Group F (high dose of vitamin E) was infected with 1.0×10^6 trypanosomes and treated with 5.0 mg of vitamin E per kilogram of body weight. The experiment lasted for 21 days after *T. b. brucei* infection was established. Blood for microscopic examinations and serum was collected weekly from the three rats across the groups and taken to Divine Chemicals and Analytical Laboratory, Nsukka, for kidney function analyses. On the last day of the experiment, one kidney 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

Trypanosoma brucei 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 0.1 ml of infected blood in 0.3-ml normal saline containing 1×10^6 trypanosomes 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 (α-tocopherol) was procured at Science Line, New Parts, Onitsha, Anambra State, Nigeria, in a powdered bottle. The working concentrations were weighed at the Department of Biochemistry, Madonna University, Elele, from the result of acute oral toxicity (LD_{50}) test of vitamin B_{12} as thus:

Mild dose: 0.5 mg/kg body weight (b.w.d.)
Enriched dose: 2.5 mg/kg b.w.d.
High dose: 5.0 mg/kg b.w.d.

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

Standard Drug

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

Clinical Determination of Renal Functions

Blood samples for clinical determination of 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 rpm 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 syringe and needle and stored in a clean sample bottle for the renal function determinations.

Urea nitrogen determination

The present method was based on the modification of the procedure of Talke and Schubert (1965).

Creatinine determination

The present procedure was based on the modification of the kinetic method (Talke & Schubert, 1965).

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 four grades of alcohol (70%, 80%, 90%, and absolute alcohol), cleared in three grades 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 three grades of xylene and rehydrated in three grades 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 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 megapixels microscope camera at ×160 and ×400 magnifications.

Statistical Analysis

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

RESULT

Biochemical Parameters

Serum urea nitrogen concentration

The result of mean urea nitrogen concentration (mg/dl) levels at short and long duration was compared in various experimental groups. On the seventh day after infection, the mean serum urea nitrogen concentration of the negative control group was significantly higher (52.941 ± 0.131) than that of the normal control (31.950 ± 0.330; $p < 0.05$), which was also week dependent (Table 1). The standard control was not significantly different (32.535 ± 0.396) when compared to the normal control ($p < 0.05$). The vitamin E-treated groups were significantly lower than the negative control group ($p < 0.05$). Only the high dose of vitamin E at Week 2 after infection returned the parameter to the level of the normal control. The mean urea nitrogen concentrations followed a similar pattern at Weeks 2 and 3 after infection and treatment.

Table 1. Effect of Tocopherol on Serum Urea Nitrogen Concentration (mg/dl)

Groups	Week 1	Week 2	Week 3
A	$31.950 \pm 0.330^{a,1}$	$32.675 \pm 0.522^{a,1}$	$31.850 \pm 0.528^{a,1}$
B	$52.941 \pm 0.131^{b,1}$	$55.701 \pm 0.483^{b,2}$	$61.250 \pm 0.727^{b,3}$
C	$32.535 \pm 0.396^{a,1}$	$32.346 \pm 0.272^{a,1}$	$32.500 \pm 0.455^{a,1}$
D	$38.053 \pm 0.406^{c,1}$	$40.840 \pm 0.392^{c,1}$	$37.920 \pm 0.352^{c,1}$
E	$38.067 \pm 0.472^{c,1}$	$40.814 \pm 0.329^{c,1}$	$37.674 \pm 0.236^{d,1}$
F	$35.627 \pm 0.280^{d,1}$	$33.118 \pm 0.268^{a,1}$	$33.031 \pm 0.276^{d,1}$

Note. In a column, mean values with the same superscript letter are not significantly different ($p > 0.05$). In a row, mean values with the same superscript number are not significantly different ($p > 0.05$).

Serum creatinine concentration

The result of creatinine level (mg/dl) on the seventh day after infection in the negative control was significantly higher (0.467 ± 0.010) than that of the normal control (0.325 ± 0.028; $p < 0.05$; Table 2). The standard control group increased significantly (0.363 ± 0.017) more than the normal control (Group A; $p < 0.05$). The groups treated with tocopherol were not significantly different when compared to

the negative control group ($p > 0.05$). There was a significant difference in serum creatinine levels in the negative control between the durations of the study ($p < 0.05$). This showed that the serum creatinine levels increased following the proliferation of the parasites. The mean creatinine levels followed a similar pattern at Weeks 2 and 3 after infection and treatment.

Table 2. Effect of Tocopherol on Serum Creatinine Concentration (mg/dl)

Groups	Week 1	Week 2	Week 3
A	$0.325 \pm 0.028^{a,1}$	$0.321 \pm 0.026^{a,1}$	$0.326 \pm 0.035^{a,1}$
B	$0.467 \pm 0.010^{b,1}$	$0.497 \pm 0.008^{b,1}$	$0.556 \pm 0.020^{b,2}$
C	$0.363 \pm 0.017^{c,1}$	$0.347 \pm 0.013^{a,1}$	$0.349 \pm 0.006^{a,1}$
D	$0.388 \pm 0.005^{c,1}$	$0.378 \pm 0.004^{c,1}$	$0.381 \pm 0.007^{c,1}$
E	$0.374 \pm 0.010^{c,1}$	$0.373 \pm 0.007^{c,1}$	$0.358 \pm 0.008^{c,1}$
F	$0.362 \pm 0.006^{c,1}$	$0.374 \pm 0.005^{c,1}$	$0.378 \pm 0.008^{c,1}$

Note. In a column, mean values with the same superscript letter are not significantly different ($p > 0.05$). In a row, mean values with the same superscript number are not significantly different ($p > 0.05$).

Histopathology of the Kidney
Normal control (Group A)

Sections of the kidney collected from the animals in this group (normal control) neither infected with *T. b. brucei* nor treated with vitamin E showed normal glomeruli (G) in their Bowman's capsules (white arrow), surrounded by myriads of normal renal tubules (proximal convoluted tubules, pars recta; distal convoluted tubules; and collecting ducts) suspended in well-vascularized connective tissue matrix (Plate 1).

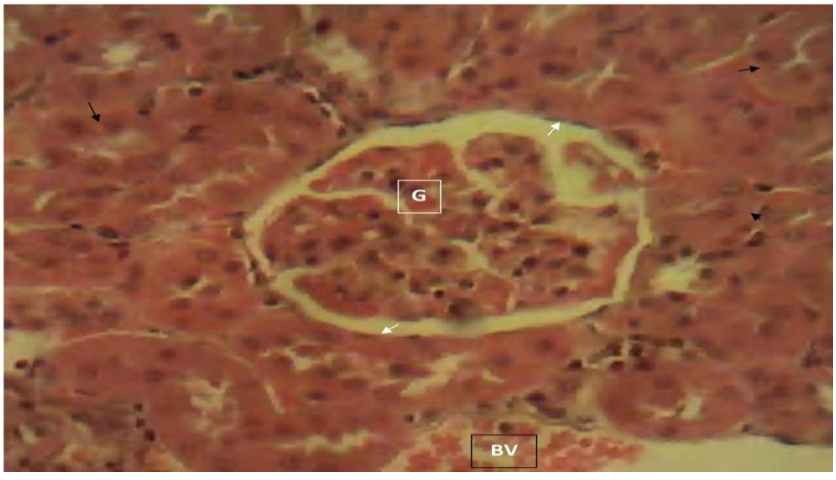

Plate 1. Photomicrograph of the transverse section of the kidney of the rat in the normal control group (Group A). Black arrow = renal tubules (H&E, ×400).

Negative control (Group B)

Sections of the kidney collected from the animals in this group (negative control) infected with 1.0×10^6 *T. b. brucei* and left untreated with vitamin E showed multifocal degeneration and necrosis of the epithelial lining cells of the renal tubules (arrow) in both the cortex and the outer medulla. The affected tubules show cytoplasmic vacuolation and nuclear pyknosis (Plate 2).

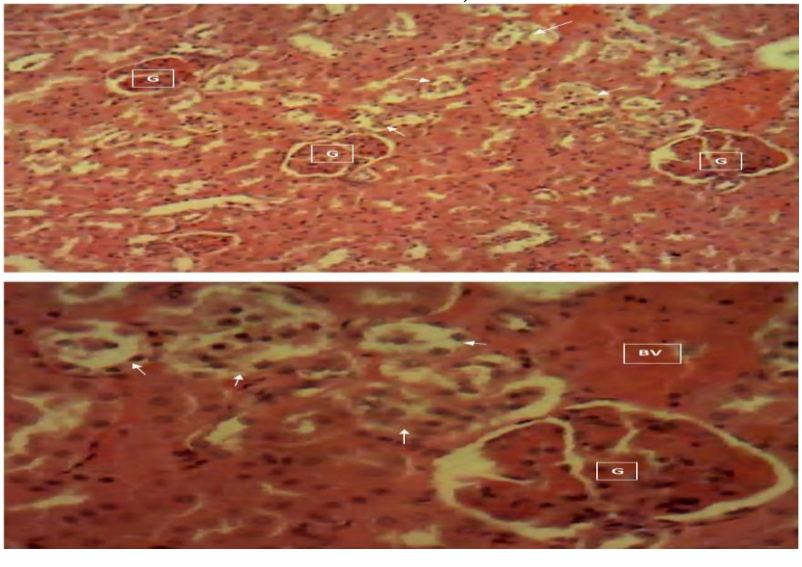

Plate 2. Photomicrograph of transverse section of kidney of rat in the negative control group (Group B). G = glomeruli (H&E, ×160 and ×400).

Standard control (Group C)

Sections of the kidney collected from the animals in this group (standard control) infected with 1.0 x 10^6 *T. b. brucei* and treated with 0.2 ml of the standard drug (diminazene aceturate) showed the normal renal histomorphology (Plate 3).

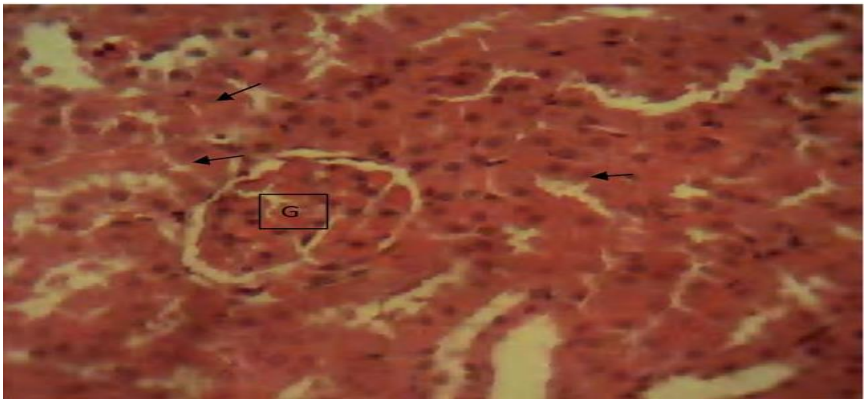

Plate 3. Photomicrograph of the transverse section of the kidney of the rat in the standard control group (Group C). G = glomeruli, arrow = renal tubules (H&E, ×160).

Low dose of vitamin E (Group D)

Sections of the kidney collected from the animals in this group (low dose of vitamin E) infected with 1.0 x 10^6 *T. b. brucei* and treated with 0.5 mg of vitamin E per kilogram of body weight showed normal renal histomorphology (Plate 4).

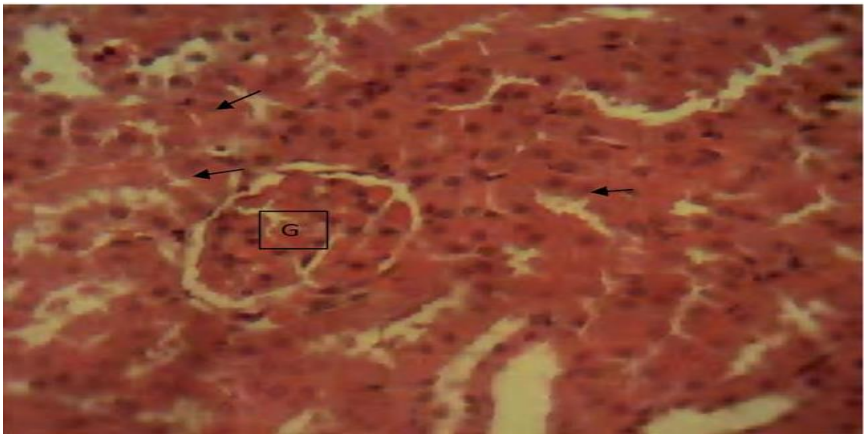

Plate 4. Photomicrograph of the transverse section of the kidney of the rat in the group with a low dose of vitamin E (Group D). G = glomeruli, arrow = renal tubules (H&E, ×400).

Medium dose of vitamin E (Group H)

Sections of the kidney collected from the animals in this group (medium dose of vitamin E) infected with 1.0 × 10^6 *T. b. brucei* and treated with 2.5 mg of vitamin E per kilogram of body weight showed normal renal histomorphology (Plate 5).

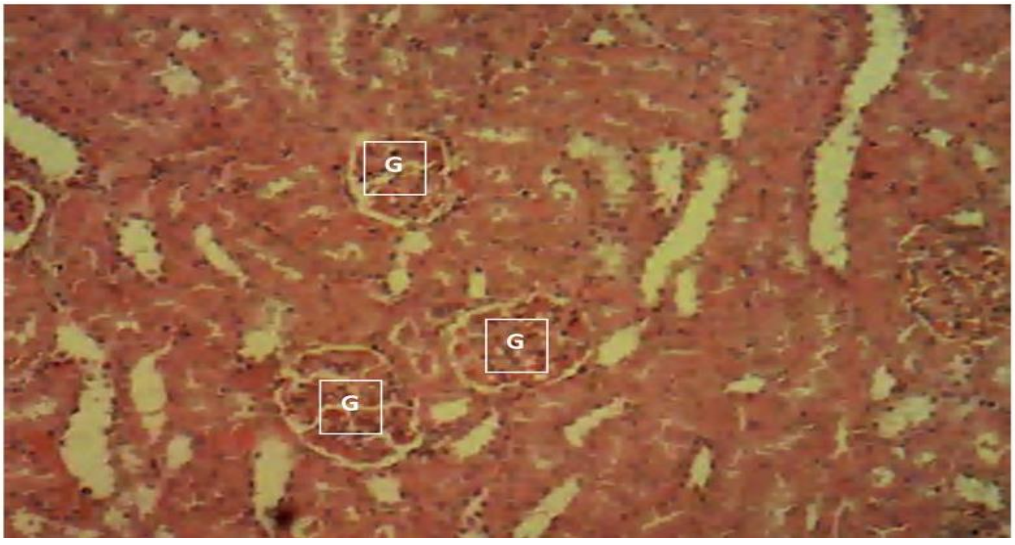

Plate 5. Photomicrograph of the transverse section of the kidney of the rat in the group with a medium dose of vitamin E (Group E). G = glomeruli, arrow = renal tubules (H&E, ×400).

High dose of vitamin E (Group F)

Sections of the kidney collected from the animals in this group (high dose of vitamin E) infected with 1.0×10^6 *T. b. brucei* and treated with 5.0 mg of vitamin E per kilogram of body weight showed normal renal histomorphology (Plate 6).

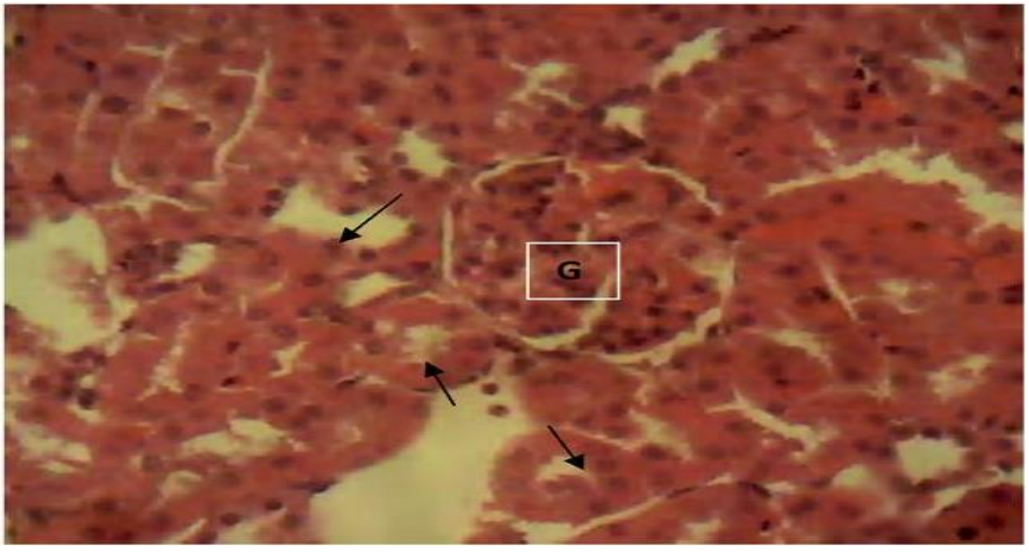

Plate 6. Photomicrograph of the transverse section of the kidney of the rat in the group with the high dose of vitamin E (Group F). G = glomeruli, arrow = renal tubules (H&E, ×400).

DISCUSSION AND CONCLUSIONS

Discussion

High serum urea level recorded in the trypanosome-infected rats aligns with the reports of Abenga et al. (2005) on infection with *Trypanosoma vivax*. The present study agrees with the result obtained by Taiwo et al. (2003), who recorded a higher concentration of urea and creatinine in sheep infected with *T. congolense* and *T. brucei*. The present study also agrees with the observation of Nguru et al. (2008), who found that urea nitrogen concentration significantly increased in the plasma of monkeys infected with *T. b. rhodesiense*. Elevated serum urea is associated with chronic kidney diseases (Lopez-Giacoman & Madero, 2015). The decrease in the trypanosome-induced urea elevation upon administration of vitamin E is suggestive that the intervention can shield the kidney from destruction by reactive oxygen species (Verhagen et al., 2006). The elevated creatinine concentration obtained in the study is consistent with the work of Allam et al. (2011), who recorded a significant increase in serum creatinine concentration in rats infected with *T. b. brucei*. Ezeokonkwo et al. (2012) linked the increased serum creatinine level to the destruction of kidney cells resulting in the inability of the kidneys to excrete creatinine. The drop in serum creatinine concentration of infected and treated rats when compared with the negative control counterparts indicates the capacity of the antioxidant vitamin E to provide some degree of protection to the kidneys during the disease. This further discusses the protective value of vitamin E in relation to trypanosomal infection. Histological lesions such as multifocal degenerations, congestion, cellular infiltration, and necrosis of the epithelial lining cells were observed in the infected and untreated rat. This is in line with the observation of Plotnikov et al. (2007). However, the groups infected and treated with vitamin E showed regeneration of the epithelial lining cells of the renal tubules suggesting antioxidant activity of vitamin E (Verhagen et al., 2006).

Histological Conclusion of the Study

The result of the present study inferred that the administration of vitamin E caused a dose-dependent reduction in the concentrations of serum urea nitrogen and creatinine level of *T. brucei brucei*-infected rats.

Ethics Statement

All animals used in this experiment were maintained in accordance with protocols approved by the institutional animal ethical committee of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The authorization was approved by the committee.

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