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Evaluation of Histopathological Effect of Kaempferol in Trypanosoma Brucei Brucei Experimentally Infected Mice

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ABSTRACT

Kaempferol is a polyphenolic compound that is widely distributed in plants. It is used in the treatment of different immunocompromised disease conditions. Toxicity and development of resistance are two major setbacks associated with avilable synthetic antitrypanosomal drugs, therefore, search for safer and more effective alternative therapy of trypanosomosis becomes paramount. This study was aimed at evaluating the histopathological effect of kaempferol in mice with experimental Trypanosoma brucei brucei infection. Thirty-six adult Swiss albino mice of either sexes were randomly divided into six groups of six mice each. Mice in group I were untreated uninfected. Mice in group II were pre-treated with kaempferol (1 mg/kg) for 14 days. Mice in groups II to VI each were inoculated with blood containing Trypanosoma brucei brucei (106 trypanosomes/ml of blood/mouse) intraperitoneally. Histopathological examination was also conducted post-infection and post-treatment. Histopathological lesions observed were acute and are seen only in infected untreated groups, which involved the brain (slight necrosis), heart (slight myocardial necrosis s) and kidney (lymphocyte hyperplasia and tubular necrosis), liver (vascular congestion), lungs (perivascular lymphocyte infiltration) and alveolar congestion). Kaempferol treated mice showed no clear histopathological lesion. Therefore, treatment with kaempferol in mice with experimental Trypanosoma brucei brucei infection have prevented the parasite from reaching the selected organs being examined.

KEYWORDS: Diminazene aceturate, Histopathology, Kaempferol, Mice, Trypanosoma brucei brucei.

ARTICLE DETAILS

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INTRODUCTION

Trypanosomosis is a disease caused by the protozoan parasite from genus Trypanosoma and transmitted by the tse- tse fly (Glossina species) and other biting flies (Masocha et al., 2012), making the incidence of the disease to be of great concern in the tropics (Anosa et al., 1983). The trypanosomes which affect both man and animals have been subdivided into two, namely the haematic groups (Trypanosoma congolense and T. vivax) which always remain in the plasma of the host's blood and the tissue invading group (T.brucei, T.evansi, T.rhodesiense, T.gambiense and T. equiperdum) which are found extravascularly and intravascularly (Anosa et al.,1977).

Diminazene aceturate is probably the most commonly used therapeutic agent for trypanosomosis in livestock in Sub-Saharan Africa (Geerts and Holmes, 1998), even in Nigeria. Complete dependence on the drug in many situations of trypanosomosis by the veterinarians has been discouraged due to their toxic effects, high cost and frequent development of resistance the parasites (Geerts and Holmes, 1998) because the drugs effectively eliminate the parasites from the blood stream and the animal appears to have recovered but later undergoes relapse infection which may be characterized by severe neurological infection leading to the death of the affected animal. In Nigeria, the occurrence of drug resistance

to available trypanocides has been attributed to the presence of fake drugs, abuse of the existing drugs and inadequate dosing of the drugs in trypanosomosis therapy (Ezeokonkwo et al., 2010). Therefore, the current challenge to the majority of African pastoralists is to optimize the use of the relatively old existing drugs (Ezeokonkwo et al., 2010). In view of this, the use of drug combinations, new therapeutic regimens and the use of Slow-Release Devices (SRD) of existing trypanocides have been suggested (Geerts and Holmes, 1998).

Secondary metabolites in plants including kaempferol are responsible for a variety of pharmacological activities (Mahomoodally et al., 2005; Pandey, 2007), Oxidant stress arises when there is an imbalance between radical-generating and radical-scavenging activities; it may, therefore, cause an increase in the formation of oxidative products as well as the pathology in some organs (Kobo et al 2014a) Infection caused by Trypanosoma brucei group of parasites have been shown to alter the antioxidant defense of the host thereby inflicting damage in some organs (Kobo et al 2014b). Kaempferol being a strong antioxidant will either mitigate or prevent the generation of free radicals (Kobo et al 2014a) such as those generated by trypanosomes (Shaba et al., 2011). Kaempferol has also been reported to have antioxidant, anti-inflammatory, antiestrogenic, cardioprotective, cancer chemo-preventive, neuroprotective, antidepressant and anxiolytic effects (Butterweck et al., 2000). In view of the therapeutic benefits of kaempferol, this study was aimed at evaluating the histopathological effect kaempferol in mice with experimental Trypanosoma brucei brucei infection.

MATERIALS AND METHODS

Location of the Research

The study was conducted in the Departments of Veterinary Pharmacology and Toxicology and Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University (A.B.U), Zaria, Kaduna State, Nigeria.

Experimental Animals

Thirty-six (36). adult Swiss albino mice of either sex weighing between 18 and 22 grams were used in this study. The mice were reared in the animal house, Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria. These animals were housed in locally fabricated mice cages at room temperature (25 ^{o}C), Wood shavings were used as beddings and changed once every two days. The experimental mice were allowed free access to rat chow and water ad-libitum.

All animal experiments were carried out according to international guidelines as approved by the postgraduate (ethical) committee of the Faculty of Veterinary Medicine, Ahmadu Bello University Zaria.

The Parasite

Trypanosoma brucei brucei was obtained from the National Veterinary Research Institute (N.V.R.I) Vom, Jos, Plateau State, Nigeria. The parasites were maintained by continuous passage in a donor mouse. Parasitaemia was monitored by use of wet mount viewed under × 400 magnifications (Herbert and Lumsden, 1976).

Drugs, Sources and Preparation

Kaempferol was sourced from whitehead scientific (pty) limited, South Africa, it came along with the following details; CAS number (520-18-3), Catalog number (3603), EC number (208-287-6) and batch number (3). Diminazene aceturate was purchased from pharmacy unit of the Veterinary Teaching Hospital (VTH).

The drugs (kaempferol and diminazene aceturate) were dissolved in distilled water and administered to each mouse according to the body weight. The concentrations of kaempferol and diminazene aceturate used were 0.5 mg/ml and 3 mg/12.5 ml, respectively.

Experimental Infection of the Mice

Trypanosomes infected blood was obtained from the tail of the infected donor mice at peak of parasitaemia (10^9) and used to maintain parasite suspension in phosphate buffer saline glucose solution, which was inoculated into peritoneal cavity of uninfected mice. The suspension contained 3 or 4 trypanosomes per microscopic field at × 100 magnification (approximately 10^6 trypanosomes per ml) as described by Ekanem and Yusuf (2008). The Thirty-six adult mice were randomly divided into six groups of six mice each and were treated as follows:

Group I- The mice in this group were neither infected with the parasites nor treated with any substance and therefore served as neutral control group.

Group II- Each of the mice in this group was pre-treated individually with kaempferol (1 mg/kg per os) for 14 days. Thereafter, each mouse was infected with Trypanosoma brucei brucei (10^6 trypanosomes/ml of blood i.p).

Group III- Each of the mice in this group was infected with Trypanosoma brucei brucei (10^6 trypanosomes/ml of blood i.p). After infection was established, each mouse was treated once with diminazene aceturate (3.5 mg/kg i.p) intraperitoneally. Animal in this group served as treated controls.

Group IV- Each of the mice in this group was infected with Trypanosoma brucei brucei(10^6 trypanosomes/ml of blood). After the establishment of infection, each mouse was treated with diminazene aceturate (3.5 mg/kg i.p) intraperitoneally, and then treated with kaempferol (1 mg/kg per os) for 9 consecutive days.

Group V- Each of the mice in this group was infected with Trypanosoma brucei brucei (10^6 trypanosomes/ml of blood i.p) and then treated with with kaempferol (1 mg/kg per os) for 9 consecutive days.

Group VI- Each of the mice in this group was infected with Trypanosoma brucei brucei (10^6 trypanosomes/ml of blood i.p) and then administered normal saline at (5 ml/kg per os) for 9 consecutive days. Animals in this group served as untreated controls.

Histopathological Evaluation

Tissue samples were collected from the brain, heart, liver, kidneys and lungs were obtained from each group. The samples were fixed in 10% formaldehyde except brain samples which was fixed in Bouin's solution. All samples were stored in labeled plastic sample bottles until processed. At processing, the tissues were embedded in paraffin wax, cut at 5µ thickness, mounted on microscope slides and stained with haematoxylineosin (H-E) as described by Drury and Wallington (1976). The prepared sections were examined under light microscope (Zeiss Microscope®, Olympus Optical Co. ltd, Japan) using x200 and x400 objectives for histopathological changes. The coloured images of organs and tissue sections revealing histopathological lesions were captured using digital camera (Model ES30, Samsung, U.K.)

Data Analysis

The severities of histopathological findings from organs were scored as Mild (+), Moderate (++), Severe (+++) lesion. Insignificant histopathological findings were scored as negative (-) and presented in tables.

RESULTS

Table 1. Histopathological Lesions Observed in the Trypanosoma brucei brucei Infected Mice

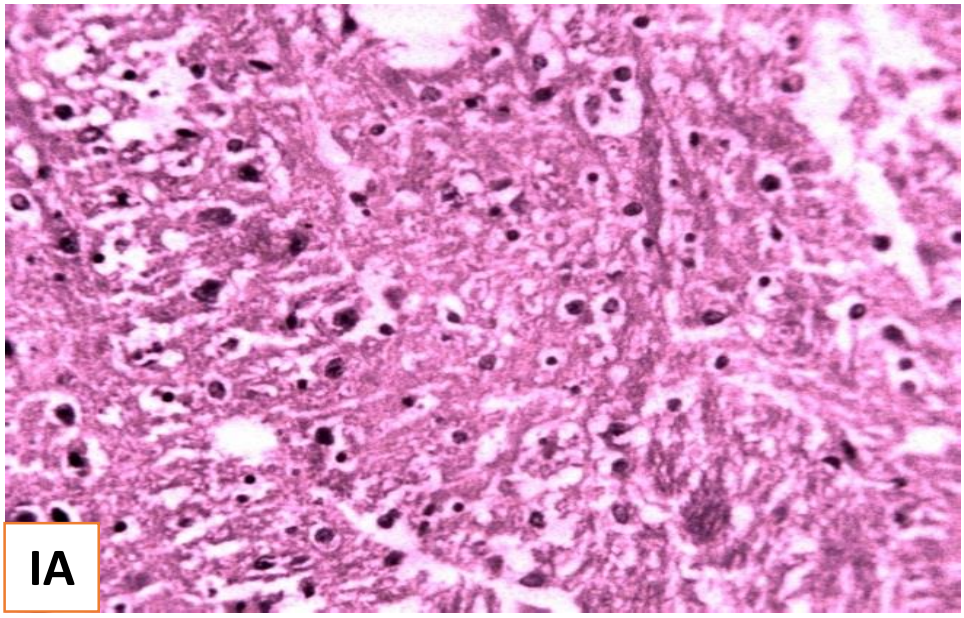
Histopathological findings of the tissue sections revealed significant lesions only in the brain, heart, kidney, liver and lungs of the infected mice at postmortem conducted on day 15 post-infection. Only infected untreated group showed significant lesions (Plates B) and are presented in this study (Table 1).

Organs showing lesion	Lesions observed	Severity of lesions in experimental group of mice (Infected1untreated)
Brain	Slight necrosis	+++
Heart	Slight myocardial necrosis	++
Liver	vascular congestion	+++
Kidney	Lymphocyte hyperplasia and tubular necrosis	+
Lungs	Perivascular lymphocyte infiltration and alveolar congestion	+

Key: Mild (+), Moderate (++), Severe (+++) lesion and negative (-).

Effect of the Treatment with Kaempferol and/or Diminazene Aceturate on Histopathology

The histopathological changes observed in the infected and untreated mice were presented in the photomicrograph; brain (slight necrosis), heart (slight myocardial necrosis) and kidney (lymphocyte hyperplasia and tubular necrosis), liver (vascular congestion), lungs (perivascular lymphocyte infiltration and alveolar congestion).



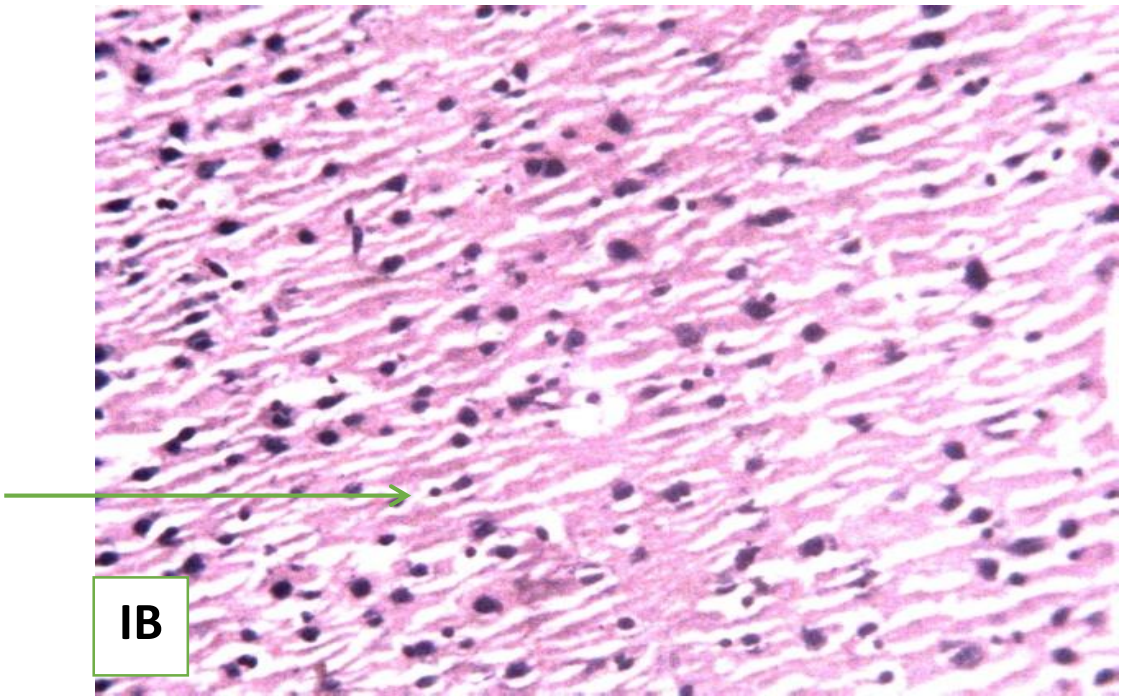
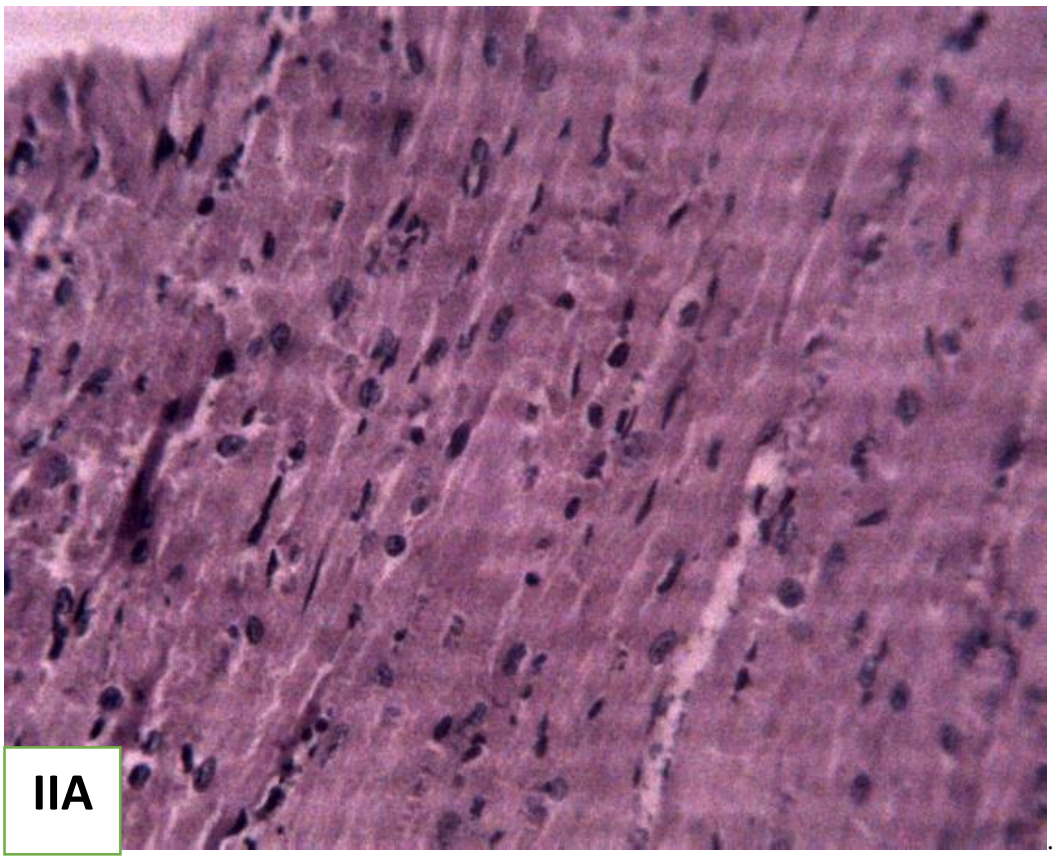


Plate I: Photomicrograph of the section of the brain of a mouse showing normal tissue (A) and slight necrosis (B) in the brain of a mouse infected with Trypanosoma brucei brucei (untreated control) (H and E) × 200



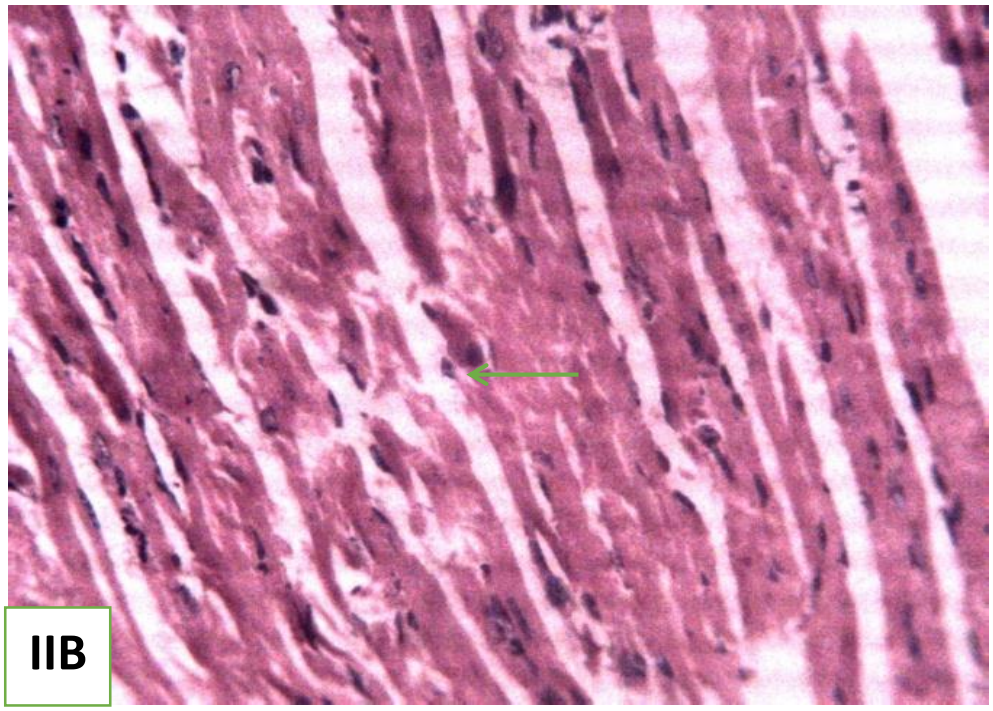
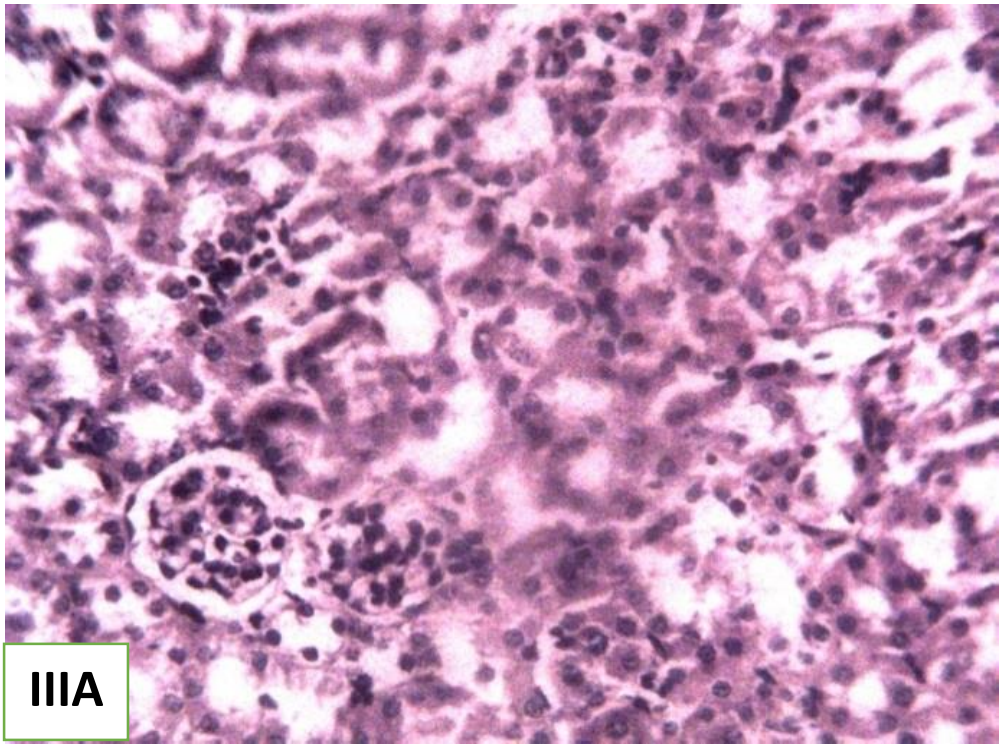


Plate II: Photomicrograph of the section of the heart of a mouse showing normal tissue (A) and myocardiac necrosis (B) in the heart of a mouse infected with Trypanosoma brucei brucei (untreated control) (H and E) × 200



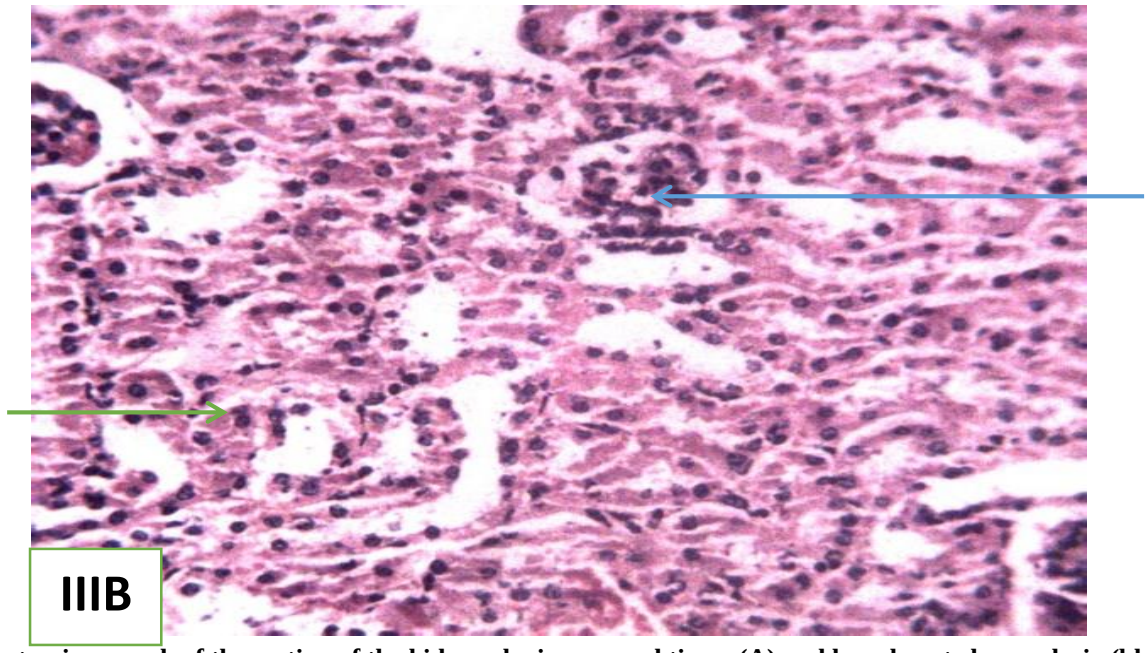
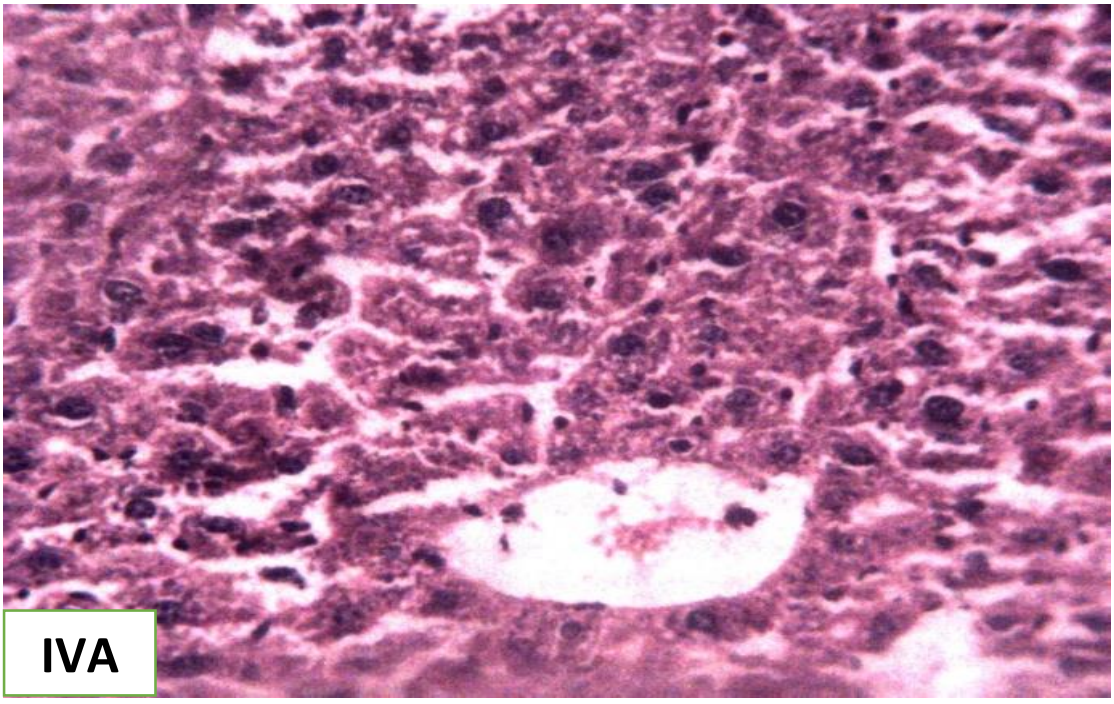


Plate III: Photomicrograph of the section of the kidney shwing normal tissue (A) and lymphocyte hyperplasia (blue arrow) and tubular necrosis (red arrows) in the kidney of a mouse infected with Trypanosoma brucei brucei (untreated control) (B) (H and E) × 200



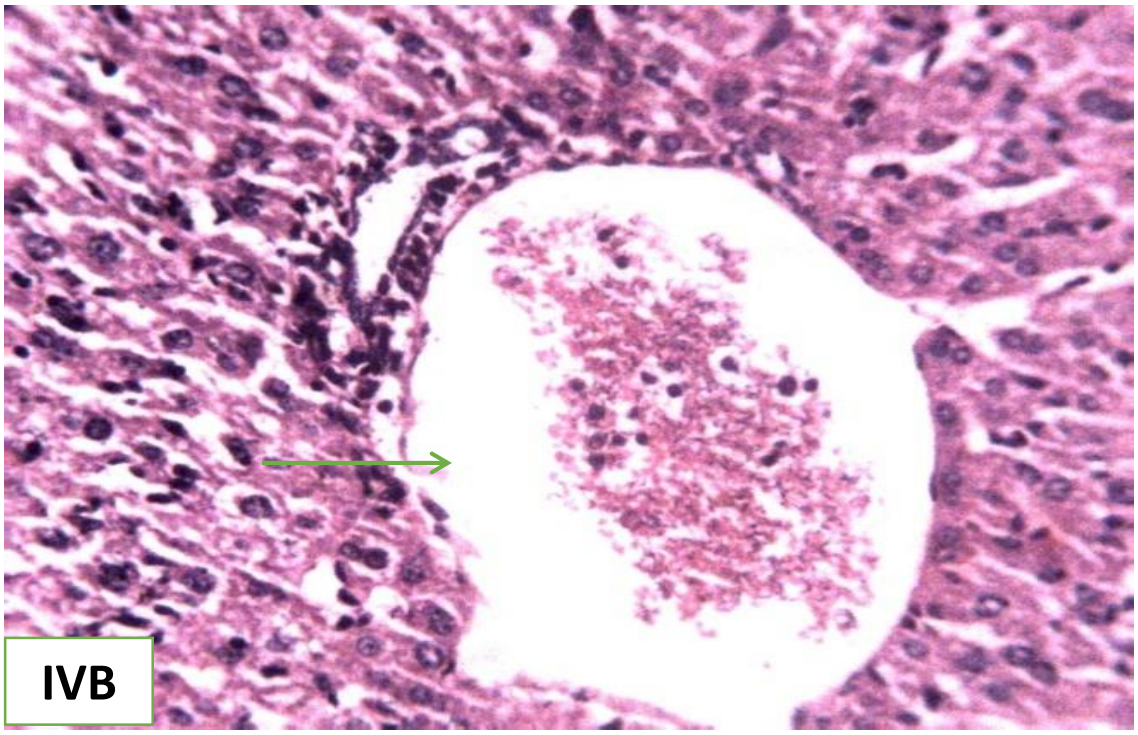
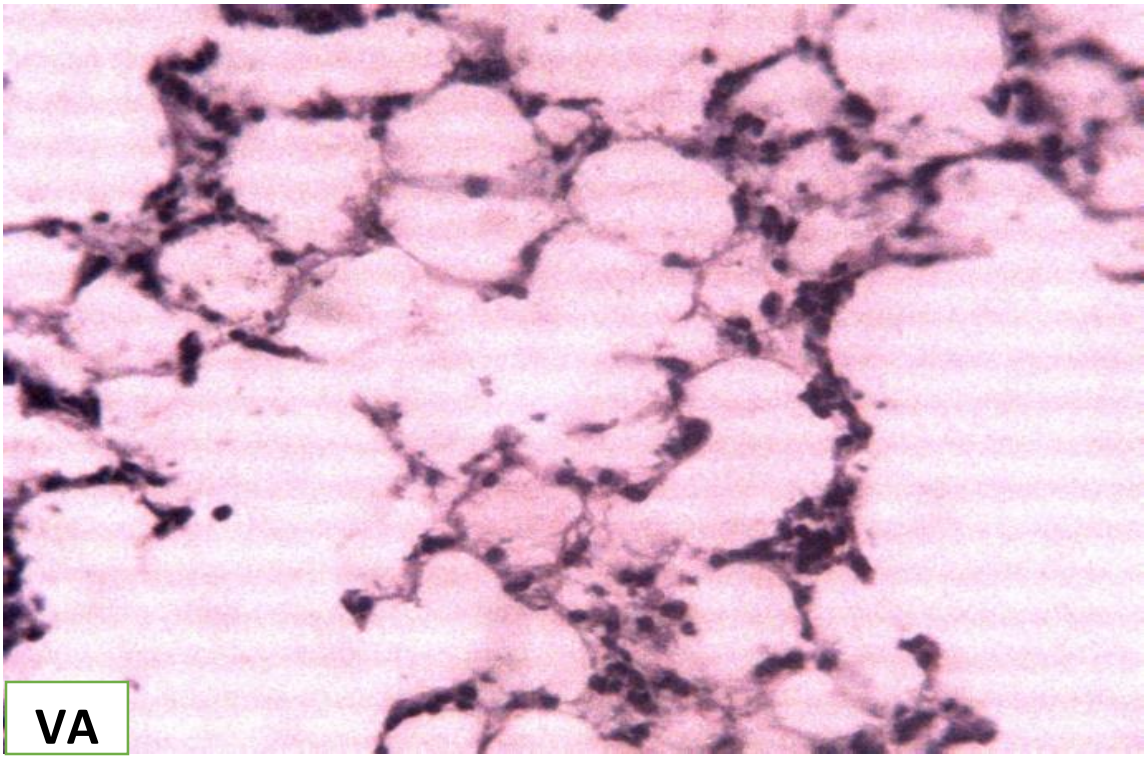


Plate IV: Photomicrograph of the section of the liver of a mouse showing normal tissue (A) and vascular congestion in the liver of a mouse infected with Trypanosoma brucei brucei (untreated control) (B) (H and E) × 200.



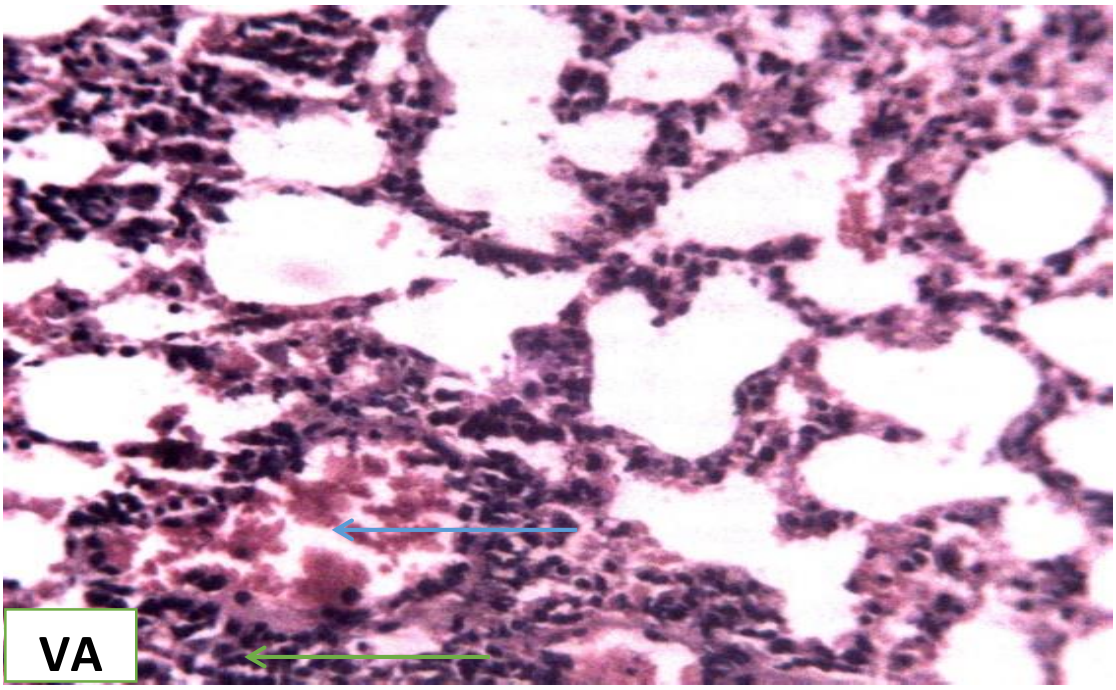


Plate V: Photomicrograph of the section of the lung of a mouse showing normal tissue (A) and perivascular lymphocyte infiltration (red arrow) and alveolar congestion (blue arrow) in the lung of a mouse infected with Trypanosoma brucei brucei (untreated control) (B) (H and E) × 200

DISCUSSIONS

In this study, the two days pre-patent period (PPP) of experimental Trypanosoma brucei brucei infection in mice agreed with earlier findings of Kobo et al., (2014a) that reported a prepatent period of exactly 2 days in mice infected with T. brucei brucei. Trypanosomosis is a disease whose pathological effects are initiated through the release of cytokines and nitric oxide (Kobo et al., 2014a). Polyphenols have been shown to attenuate cytokine and nitric oxide-induced inflammation (Karina et al., 2011) probably due to their reactive oxygen species (ROS) scavenging ability (Kobo et al., 2014b). Therefore, we may speculate that the ability of the kaempferol to increase the survival of mice in this study may be due to their ability to aid antioxidant defense system and reduce oxidative stress by protecting the defense system against the damaging effects of ROS. The survival time of mice treated with diminazene aceturate was significantly increased compared to the untreated control (Kobo et al., 2014a). This may be due to the ability of the drug to eliminate the parasites from the blood (Saba et al.,2007).

The histopathological findings in Trypanosoma brucei brucei infected, untreated mice at termination of this experiment on day 9 were; brain slight necrosis due to mild neuroinflammation and encephalitis (Jean et al., 2019). Neuroinflammation reaction is recognized as characteristic CNS stage in trypanosomosis and has been noted in additional description produced from fatal cases (Mott, 1911), because the inflammatory cell infiltrate consists of predominantly mononuclear lymphocytes together with large number of plasma cell (Jean et al., 2019).

slight myocardial necrosis observed in the heart was due to presence of the parasites within the interstitium in close proximity to cardiomyocytes (McCarrol et a., 2015), thereby resulting in high-rate inflammation that are linked with products released by the parasites. The inflammation characterized by degeneration of myocardial fibers with decreased mitochondrial size and myofibril contents, fragmentation of degenerating fibers as well as accumulation of inflammatory cells.

The observed lymphocyte hyperplasia and tubular necrosis in the kidney was due to glomerulonephritis characterized by deposition of electron dense materials along the basement membrane and epithelium of bowman's capsule.

Vascular congestion in the liver was due to deposits of free iron from severe intravascular hemolysis, RBC sequestration in the red pulp area and cells lysis by Trypanosomes lymphotoxins also contribute to iron deposits in tissues (Garba, et al., 2017). Depletion of lymphocytes at germinal centres, severe hepatic congestion, the moderate forms of hepatic focal areas of necrosis and mononuclear cellular infiltrations are the prominent lesions also reported from other parts of the world in laboratory animals with trypanosomosi (Garba, et al., 2017). Acute forms of perivascular lymphocyte infiltration and alveolar congestion due to vascular stasis in lungs.

CONCLUSION

It was concluded that treatment with kaempferol in mice with experimental Trypanosoma brucei brucei infection have prevented the parasite from reaching tissues but showed mild signs including fever and weakness. Tissue invasion were seen only in the infected untreated mice.

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