

# Chemopreventive effect of methanolic extract of *Azadirachta indica* on experimental *Trypanosoma brucei* induced oxidative stress in dogs

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## ABSTRACT

**Introduction:** The medicinal properties of *Azadirachta indica* have been harnessed for many years in the treatment of many diseases in both humans and animals. **Materials and Methods:** Twenty-five apparently healthy dogs weighing between 3 and 8 kg were randomly divided into five groups with five dogs in each group. Ameliorative effect of *A. indica* on erythrocyte antioxidant status and markers of oxidative stress were assessed. Liver and kidney function tests were also performed. **Results:** Pre-treatment with methanolic extract of *Azadirachta indica* (MEAI) at different doses did not significantly alter the values of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activity in *Trypanosoma brucei* infection. Although, serum creatinine significantly ( $P < 0.05$ ) decreased with pre-treatment with 50 mg/kg *A. indica*, after 2 weeks of *T. brucei* infection. However, the reduced glutathione (GSH) content of the erythrocyte increased significantly in animals pre-treated with 50 mg/kg and 200 mg/kg of *A. indica* respectively. Markers of oxidative stress such as malondialdehyde and hydrogen peroxide generated were higher in animals infected with *T. brucei* with no significant ( $P > 0.05$ ) difference compared to the values obtained in pre-treated animals. Pre-treatment with 100 mg/kg and 200 mg/kg of *A. indica* significantly ( $P < 0.05$ ) decreased serum myeloperoxidase activity at 2 weeks post-infection with *T. brucei*. **Conclusion:** From this study, MEAI showed significant ability to attenuate oxidative stress and inflammation during experimental *T. brucei* infection.

**Key words:** Antioxidant enzymes, *Azadirachta indica*, Inflammation, Oxidative stress, *Trypanosoma brucei*

## INTRODUCTION

Trypanosomiasis in dogs within South Western Nigeria is often caused by *Trypanosoma brucei*.<sup>[1]</sup> Mixed infection within one out of three dogs with trypanosomiasis have been reported in *T. congolense*.<sup>[2]</sup> The acute form of the disease is the most prevalent and manifests as pyrexia, myocarditis, myositis, corneal opacity, orchitis, pedal edema and occasionally neurological signs may be present.<sup>[3]</sup> The gross and histopathological changes in the liver and kidney among other organs have been shown to indicate the degenerative effect of trypanosomes on these organs.<sup>[4]</sup> Malondialdehyde (MDA) which is a marker of oxidative

stress has been reported in *T. brucei* infection, and MDA concentration was found to increase as the infection progressed.<sup>[5]</sup>

Several medicinal properties which include anthelmintic, antiseptic, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, anticarcinogenic, diuretic, immunomodulatory, anti-inflammatory, hypoglycemic and purgatory have been attributed to *A. indica*.<sup>[6,7]</sup> In addition to these, the plant has also been reported to reduce total cholesterol, triglycerides and total lipid in serum of diabetics.<sup>[7]</sup>

Extracts of *A. indica* have been reported to produce a dose-dependent effect at delaying onset of *T. brucei rhodesiense* appearance in mice circulation and decreased the level of parasitemia. Treatment with 1000 mg/kg of plant extract was comparable to and in some cases more effective than suramin, a known trypanocidal drug.<sup>[8]</sup> Similarly, oil from *A. indica* has also been reported

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to improve liver function, detoxifies the blood and balance blood sugar levels and other chemopreventive potentials.<sup>[9,10]</sup>

The drugs derived from medicinal plants have been reported to be effective and safe against trypanosome in endemic areas.<sup>[11,12]</sup> Increasing evidence has shown that oxidative stress plays an important etiologic role in the pathogenesis of African sleeping sickness.<sup>[13,14]</sup> However, oxidative stress is due to an imbalance between reactive oxygen species (ROS) and antioxidant defense system activities with a concomitant increase in the formation of oxidative products.<sup>[15,16]</sup> Furthermore, infection caused by *T. brucei* group of parasites have been shown to alter the antioxidant defense of the host and that pathological processes are initiated through the release of cytokines and nitric oxide.<sup>[17,18]</sup>

Considering the fact that oxidative stress is a key pathophysiological mechanism in a lot of disease conditions including trypanosomiasis and that *A. indica* could be a good source of alternative medicine in developing countries that are ravaged by poverty. This study was designed to investigate the possible effects of methanolic extract of *Azadirachta indica* (MEAI) on trypanosome brucei induced oxidative stress in rats and some markers hepatic and renal dysfunction.

## MATERIALS AND METHODS

### Methanolic extract of *Azadirachta indica*

*Azadirachta indica* leaves were plucked from the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The leaves were air-dried at room temperature and subsequently powdered with an electric blender. Plant extraction was carried out at the Nigerian Institute of Science Laboratory Technology, Ibadan, Nigeria. A volume of 1 kg powdered of *A. indica* leaves was soaked in 1 L of methanol in a glass container for 72 h. The crude extract was collected from the solvent (methanol) and thereafter, filtered and concentrated using a rotary evaporator. The concentration of the extract is thus 1 mg/mL. The extract was stored at 4°C in refrigerator prior use.

### Chemicals

1,2-dichloro-4-nitrobenzene, thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium hydroxide, xylenol orange (XO), potassium hydroxide, reduced glutathione (GSH), O-dianisidine, and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and were obtained from British Drug Houses (Poole, Dorset, UK).

### Care of animals

All of the animals received humane care according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health. The ethics regulations were followed in accordance with national and institutional guidelines for the protection of the animals' welfare during experiments.<sup>[22]</sup>

### Experimental infection with the parasite (*Trypanosoma brucei*)

Single dose of 0.3 mL of *T. brucei* with three parasites per microscopic field was injected intraperitoneally into experimental animals.

### Experimental animals

Twenty-five specific pathogen free dogs weighing between 3 and 8 kg were randomly divided into five groups with five dogs in each group. These were reared in the experimental animal unit, Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

- Group I: Not infected and not treated
- Group II: Infected with *T. brucei*
- Group III: Infected with *T. brucei* and pretreated with 50 mg/kg
- Group IV: Infected with *T. brucei* and pretreated with 100 mg/kg
- Group V: Infected with *T. brucei* and pretreated with 200 mg/kg

The dogs in Groups III-V were pretreated with daily oral administration of *A. indica* (MEAI) extract for 1-week after which they infected with *T. brucei*.

### Serum collection

Blood was collected from each dog into sterile plain tubes and left in a tilted position for about 30 min to clot. It was centrifuged at 3000 rpm for 5 min. Serum was decanted into eppendorf tubes and stored at 4°C till required.

### Isolation of erythrocyte

About 5 mL of blood was collected from the jugular vein inside a heparinized bottle. The erythrocytes were washed with ice-cold phosphate buffer saline (pH 7.4) and centrifuged thrice at 4000 rpm for 10 min. The erythrocyte membrane was lysed and then re-suspended in PBS at 1:10 dilutions until the time of use to obtain ghost erythrocytes according to the method of Steck and Kant.<sup>[23]</sup>

### Biochemical assays

#### Makers of oxidative stress

Protein concentrations were determined by means of the described by Gornal *et al.*<sup>[24]</sup> Briefly, 1 mL of diluted serum

was added to 3 mL of the buret reagent. The reaction mixture was incubated at room temperature for 30 min. The mixture was thereafter read with a spectrophotometer at 540 nm using distilled water as blank. The final value for total protein was extrapolated from total protein standard curve. MDA was determined according to the method of Varshney and Kale.<sup>[20]</sup> To 1.6 mL of Tris-KCl, 0.5 mL of 30% TCA, 0.4 mL of sample and 0.5 mL of 0.75% TBA prepared in 0.2 M HCl were added. The reaction mixture was incubated in the water bath at 80°C for 45 min, cooled on ice and centrifuged at 4000 rpm for 15 min. The absorbance was measured against a blank of distilled water at 532 nm. Lipid peroxidation in units/milligram protein was calculated with a molar extinction coefficient of  $1.56 \times 10^5$  M/cm.

Hydrogen peroxide generation was determined by Woff's method  $\times$  s method.<sup>[20]</sup> To 2.5 mL of 0.1M potassium phosphate buffer (pH 7.4), 250  $\mu$ L of Ammonium ferrous sulphate, 100  $\mu$ L of sorbitol, 100  $\mu$ L of XCl, 25  $\mu$ L of H<sub>2</sub>SO<sub>4</sub> and 50  $\mu$ L of Sample was added. The mixture was mixed thoroughly by vortexing till it foam and a light pink color of the reaction mixture was observed. The reaction mixture was subsequently incubated at room temperature for 30 min. The absorbance at 560 nm using distilled water as blank. The H<sub>2</sub>O<sub>2</sub> generated was extrapolated from H<sub>2</sub>O<sub>2</sub> standard curve.

#### Antioxidant defense system

Catalase (CAT) activity was determined according to the method of Sinha.<sup>[21]</sup> Briefly, 1 mL portion from the reaction mixture (2 mL of H<sub>2</sub>O<sub>2</sub> solution, 2.5 mL of 0.01M potassium phosphate buffer (pH 7.0) and 1 mL of properly diluted sample) was blown into 1 mL dichromate/acetic acid solution by a gentle swirl at room temperature at 60 s interval for 3 min into four sets of tubes. The mixture was incubated in the water bath at 100°C for 10 min. The absorbance was read at 570 nm using distilled water as blank. One unit of CAT activity represents the amount of enzyme required to decompose 1  $\mu$ mol of H<sub>2</sub>O<sub>2</sub>/min.

Superoxide dismutase (SOD) was determined by the method of Misra and Fridovich<sup>[22]</sup> with slight modification from our laboratory. Briefly, 50 mg of epinephrine was dissolved in 100 mL distilled water and acidified with 0.5 mL concentrated hydrochloric acid. This preparation prevents oxidation of epinephrine and is stable for 4 weeks. A volume of 10  $\mu$ L of erythrocyte extract was added to 2.5 mL 0.05 M carbonate buffer (pH 10.2) followed by the addition of 300  $\mu$ L of 0.0 mM adrenaline. The increase in absorbance at 480 nm was monitored every 30 s for 150 s. The one unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during

Reduced glutathione (GSH) was estimated by the method of Jollow *et al.*<sup>[23]</sup> Briefly, 0.5 mL of 4% sulfosalicylic acid (precipitating agent) was added to 0.5 mL of sample and centrifuged at 4000 rpm for 5 min. To 0.5 mL of the resulting supernatant 4.5 mL of Ellman's reagent (0.04 g of DTNB in 100 mL of 0.1M phosphate buffer, pH 7.4) was added. The absorbance was read at 412 nm against distilled water as blank.

Glutathione peroxidase (GPX) activity was also measured according to Beutler *et al.*<sup>[24]</sup> The reaction mixtures contain 0.5 mL of potassium phosphate buffer (pH, 7.4), 0.1 mL of Sodium azide, 0.2 mL of GSH solution, 0.1 mL of H<sub>2</sub>O<sub>2</sub>, 0.5 mL of sample and 0.6 mL of distilled water. The mixture was incubated in the water bath at 37°C for 5 min and 0.5 mL of TCA was added and centrifuged at 4,000 rpm for 5 min. A volume of 1 mL of the supernatant was taken and added 2 mL of K<sub>2</sub>PHO<sub>4</sub> and 1 mL of Ellman's reagent. The absorbance was read at 412 nm using distilled water as blank.

#### Marker of inflammation

Myeloperoxidase (MPO) as a marker of inflammation was measured according to the method of Xia and Zweier.<sup>[11]</sup> 2,000  $\mu$ L of O-dianisidine and H<sub>2</sub>O<sub>2</sub> mixture was pipetted in the cuvette and 70  $\mu$ L of sample (serum) was subsequently added to it. The reaction mixture was read at 0 second, 30 seconds and 60 seconds respectively at 460 nm wavelengths. One unit of MPO activity can be defined as the quantity of enzyme able to convert/degrade 1  $\mu$ mol of hydrogen peroxide to water in one minute at room temperature.

#### Liver function tests

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase activity (ALP), urea and creatinine were estimated using Randox kits according to the manufacturer's instruction.

## RESULTS

#### The erythrocyte and serum total protein status

There was no significant ( $P > 0.05$ ) difference in the values of total protein in all the groups pretreated with MEAI after 2 weeks of infection with *T. brucei* [Figures 1 and 2]. However, there was a significant ( $P < 0.05$ ) increase in the values of total protein of erythrocyte in animals pretreatment with 200 mg/kg of MEAI after 4 weeks of infection with *T. brucei* [Figure 1]. Surprisingly, there was also a significant ( $P < 0.05$ ) decrease in serum total protein in animals pretreatment with MEAI (100 mg/kg and 200 mg/kg) after 4 weeks of infection with *T. brucei* [Figure 2].

#### The serum liver function tests

Dogs pretreated with MEAI (50, 100 and 200 mg/kg) did not show any significant ( $P > 0.05$ ) difference in

the values of serum ALT, ALP and AST after 1-week of administration [Figures 3-5]. Similarly, also, there was no significant ( $P > 0.05$ ) difference in the values of ALT, ALP, AST after 2 weeks and 4 weeks of *T. brucei* infection [Figures 3-5].

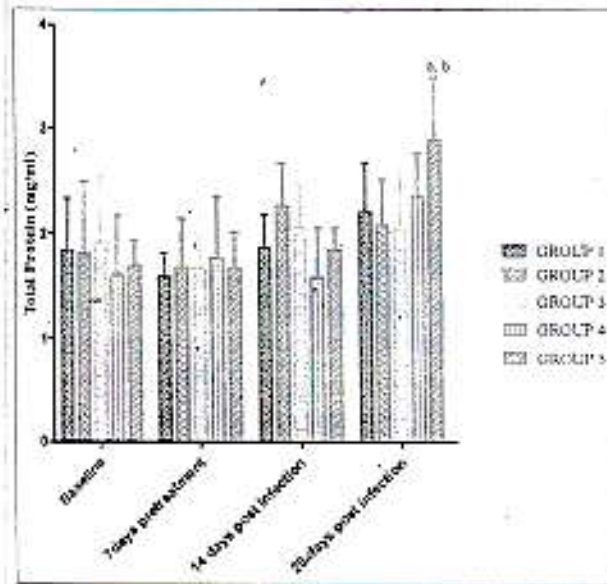


Figure 1: Effect of AI on erythrocyte TP in experimental *T. brucei* infection. values are presented as mean  $\pm$  standard deviation. Superscript (a) indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) indicates statistically significant when groups III, IV and V are compared with group II

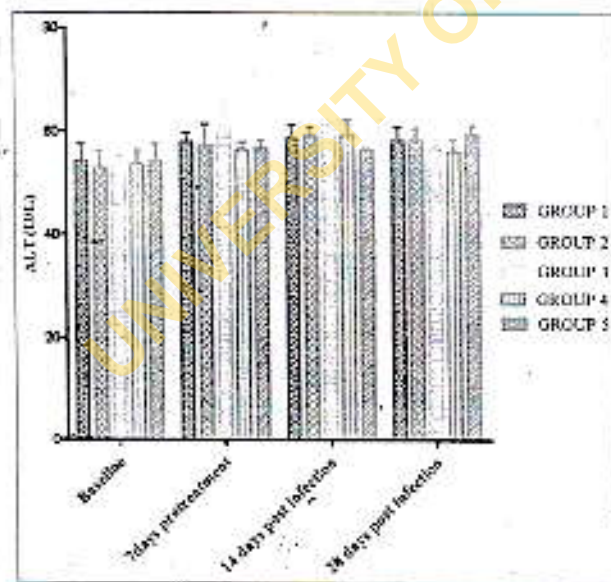


Figure 3: Effect of AI on ALT in experimental *T. brucei* infection. Values are presented as mean  $\pm$  standard deviation. Superscript (a) indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) indicates statistically significant when groups III, IV and V are compared with group II

The erythrocyte antioxidant status and markers of oxidative stress.

Pretreatment of dogs with MEAI led to no significant ( $P > 0.05$ ) difference in the markers of oxidative stress (MDA and  $H_2O_2$ ) after 1-week of administration at different dosages [Figures 6 and 7]. In addition, there was no significant ( $P > 0.05$ ) difference in the values of MDA

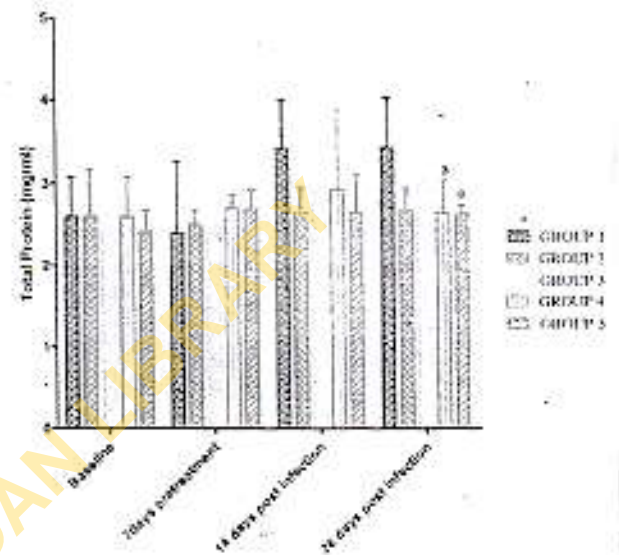


Figure 2: Effect of AI on serum TP in experimental *T. brucei* infection. values are presented as mean  $\pm$  standard deviation. Superscript (a) indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) indicates statistically significant when groups III, IV and V are compared with group II

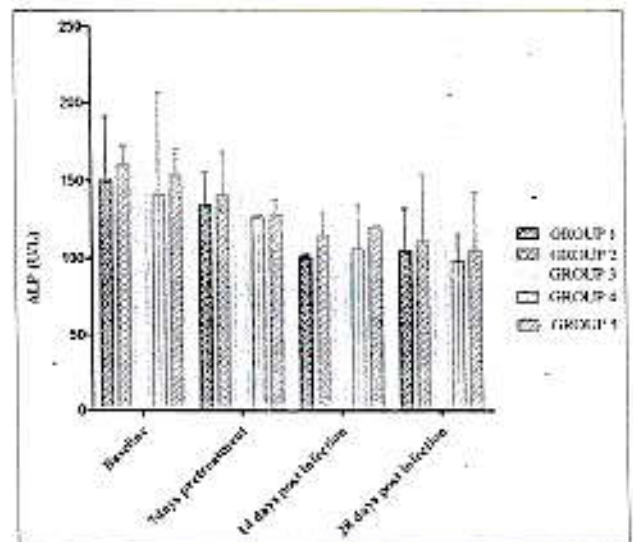


Figure 4: Effect of AI on ALP in experimental *T. brucei* infection. Values are presented as mean  $\pm$  standard deviation. Superscript (a) indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) indicates statistically significant when groups III, IV and V are compared with group II

and  $H_2O_2$  in the groups pretreated with MEAI after 2 weeks and 4 weeks of with *T. brucei* infection [Figures 6 and 7]. The GSH content was significantly ( $P < 0.05$ ) increase in animals pretreated with MEAI at (50 mg/kg and 200 mg/kg) after 2 weeks of infection [Figure 8]. However, there was no observable difference in the values of GSH content after 4 weeks of infection [Figure 8] in dogs pretreated with AI extract at different doses [Figure 8]. There was no significant ( $P > 0.05$ ) difference in the values of

antioxidant enzymes (CAT, SOD and GPX) in the groups pretreated with MEAI extract after 2 weeks of *T. brucei* infection [Figures 9-11] whereas the activity of these enzymes decrease but not significantly different after 4 weeks of *T. brucei* infection [Figures 9 and 11].

The erythrocyte and serum inflammatory status: Myeloperoxidase values in serum and erythrocyte were significantly ( $P < 0.05$ ) increased in *T. brucei* infected group

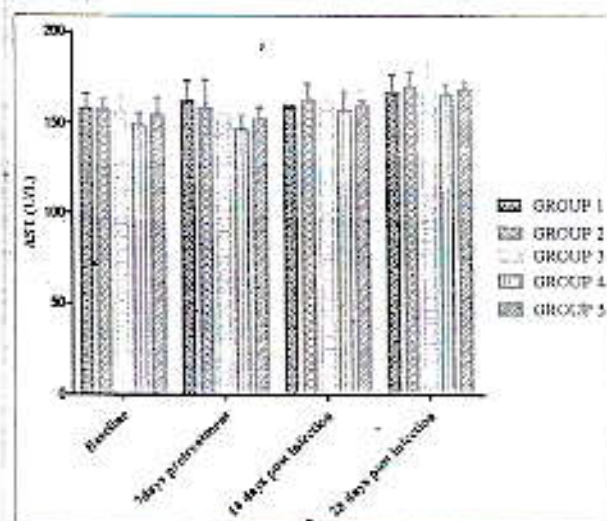


Figure 5: Effect of AI on AST in experimental *T. brucei* infection. Values are presented as mean  $\pm$  standard deviation. Superscript (a) Indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) Indicates statistically significant when groups III, IV and V are compared with group II

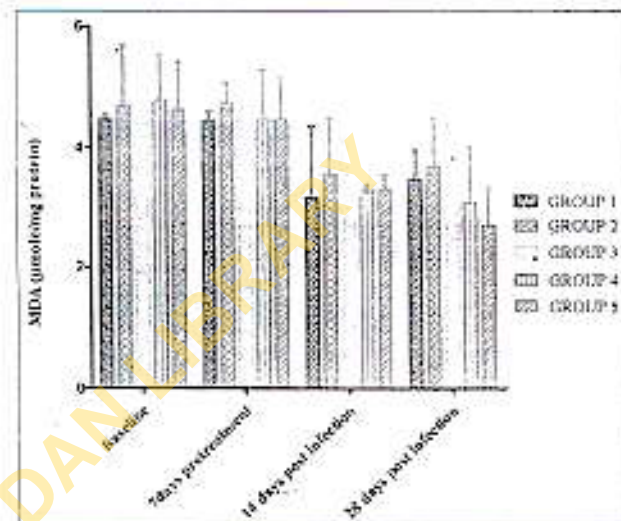


Figure 6: Effect of AI on MDA in experimental *T. brucei* infection. Values are presented as mean  $\pm$  standard deviation. Superscript (a) Indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) Indicates statistically significant when groups III, IV and V are compared with group II

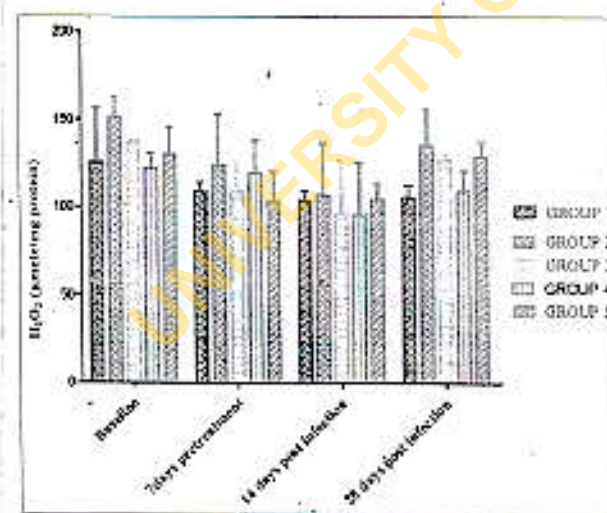


Figure 7: Effect of AI on  $H_2O_2$  in experimental *T. brucei* infection. Values are presented as mean  $\pm$  standard deviation. Superscript (a) Indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) Indicates statistically significant when groups III, IV and V are compared with group II

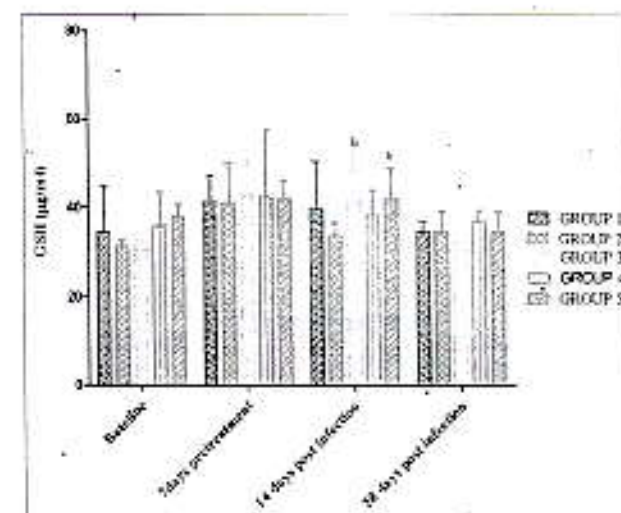
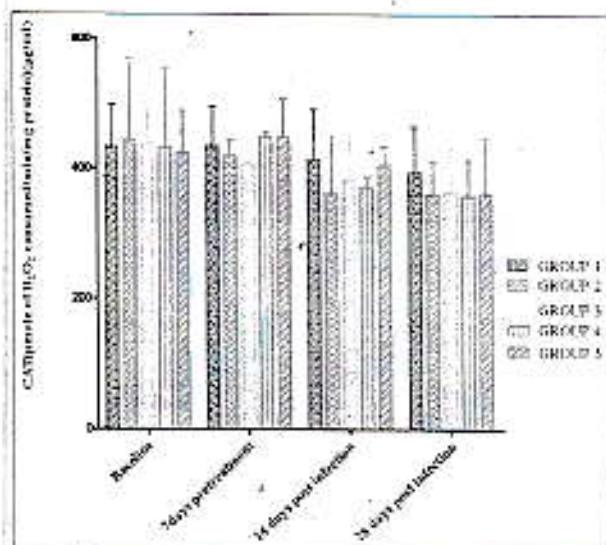
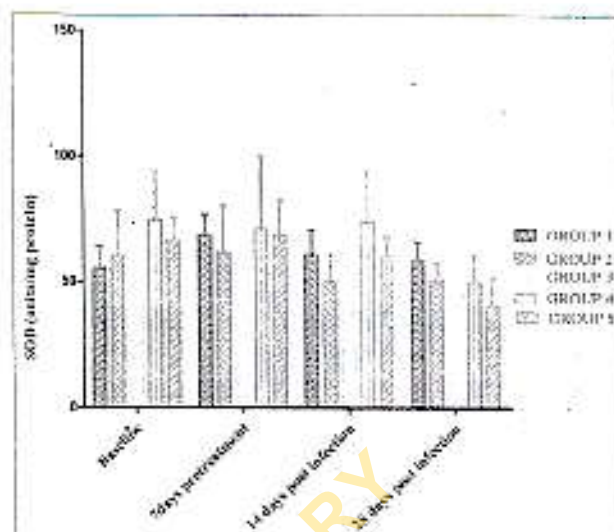


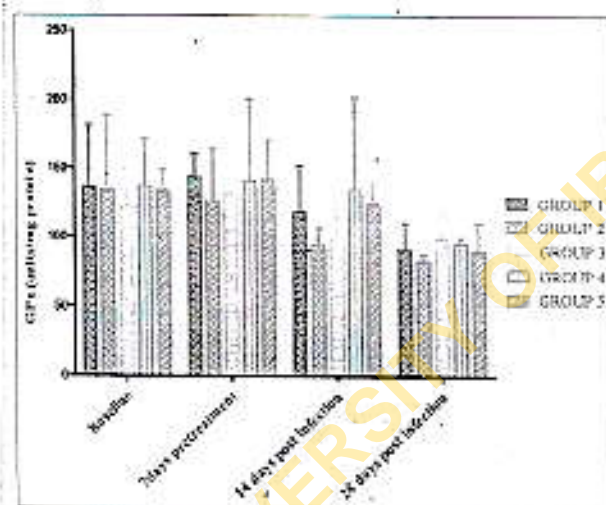
Figure 8: Effect of AI on GSH in experimental *T. brucei* infection. Values are presented as mean  $\pm$  standard deviation. Superscript (a) Indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) Indicates statistically significant when groups III, IV and V are compared with group II



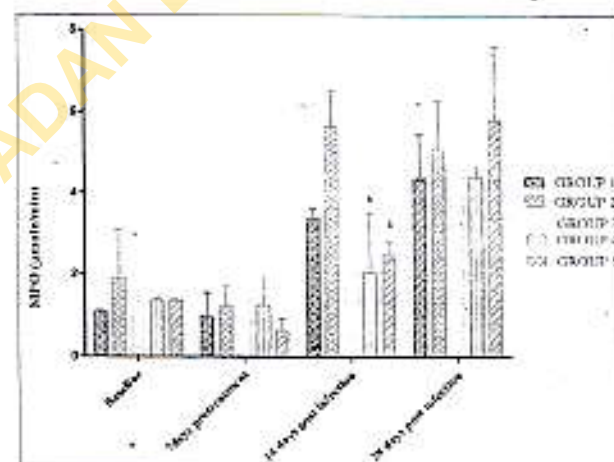
**Figure 9:** Effect of AI on Catalase in experimental *T. brucei* infection. Values are presented as mean  $\pm$  standard deviation. Superscript (a) indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) indicates statistically significant when groups III, IV and V are compared with group II



**Figure 10:** Effect of AI on SOD in experimental *T. brucei* infection. Values are presented as mean  $\pm$  standard deviation. Superscript (a) indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) indicates statistically significant when groups III, IV and V are compared with group II



**Figure 11:** Effect of AI on GPx in experimental *T. brucei* infection. Values are presented as mean  $\pm$  standard deviation. Superscript (a) indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) indicates statistically significant when groups III, IV and V are compared with group II

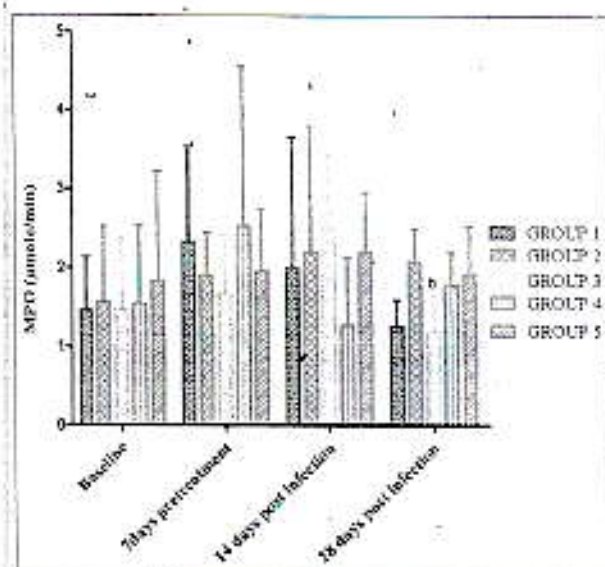


**Figure 12:** Effect of AI on serum MPO in experimental *T. brucei* infection. Values are presented as mean  $\pm$  standard deviation. Superscript (a) indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) indicates statistically significant when groups III, IV and V are compared with group II

after 2 weeks of the experiment [Figures 12 and 13]. There was also a significant ( $P < 0.05$ ) decrease in MPO activity in animals pretreated with 100 mg/kg and 200 mg/kg of MEAI after 2 weeks and 4 weeks of *T. brucei* infection [Figures 12 and 13].

The serum creatinine and blood-urea nitrogen status. There was no significant ( $P > 0.05$ ) difference in the values of blood urea nitrogen (BUN) in all the

groups pretreated with MEAI and infected with *T. brucei* [Figure 14]. There was a significant ( $P < 0.05$ ) increase in serum creatinine concentration after 2 weeks of *T. brucei* infection [Figure 15]. There was also a significant ( $P < 0.05$ ) decrease in serum creatinine values in dogs pretreated with 50 mg/kg MEAI whereas 200 mg/kg of MEAI caused a significant ( $P < 0.05$ ) increase serum creatinine values after 2 weeks of *T. brucei* infection [Figure 15]. At 4 weeks of infection of *T. brucei*, there was no significant ( $P > 0.05$ ) difference in the values



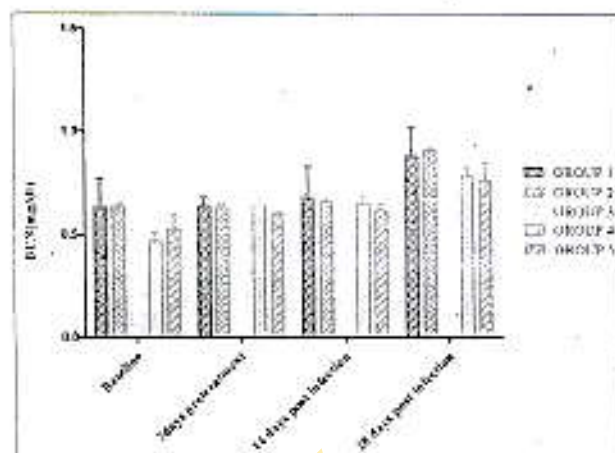
**Figure 13:** Effect of AI on erythrocyte MPO in experimental *T. brucei* infection. Values are presented as mean  $\pm$  standard deviation. Superscript (a) Indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) Indicates statistically significant when groups III, IV and V are compared with group II

of BUN and creatinine in all the groups pretreated with MEAI [Figures 14 and 15]. Combining all, pretreatment with *A. indica* modulated antioxidant enzymes, reduced oxidative stress and inflammatory response induced by *T. brucei* infection in dogs.

## DISCUSSION

In this study, there was no significant difference in MDA,  $H_2O_2$  generated, reduced glutathione (GSH), and in the activities of CAT, SOD, GPX and MPO after 7 days of oral administration of *A. indica* at different dosages. In addition, the activities of ALT, AST, ALP, and serum contents of creatinine and BUN were not significantly affected after 7 days of oral administration of *A. indica*. This indicates that *A. indica* did not induce oxidative stress and deplete the antioxidant system in this study. However, it has been shown that whole plant extract of *A. indica* exhibited improved bioavailability and lower toxicity.<sup>[52]</sup>

Trypanosomiasis was reported to induce oxidative stress and deplete antioxidants whereas exogenous antioxidants supplement, such as Vitamin C and dimethyl sulfoxide gave a better clinical improvement and survival than diminazene (a known chemopreventive drug) in *T. brucei* infection in rats.<sup>[53]</sup> This has been further buttressed by a significant increase in erythrocyte osmotic fragility and MDA concentration in *T. brucei brucei* infected Wistar rats.<sup>[54]</sup> This demonstrates the chemoprotective effects of *A. indica* on oxidative stress via decrease in lipid peroxidation and



**Figure 14:** Effect of AI on BUN in experimental *T. brucei* infection. Values are presented as mean  $\pm$  standard deviation. Superscript (a) Indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) Indicates statistically significant when groups III, IV and V are compared with group II

enhancement of the antioxidant status.<sup>[55]</sup> MDA is an end product of the oxidative degradation of lipids. In this study, there was a transient increase in MDA and  $H_2O_2$  as markers of oxidative stress in the group infected with *T. brucei*. These markers (MDA and  $H_2O_2$ ) decreased after 4 weeks of infection. This result pointed to the fact that, the infection lasted for 2 weeks and thereafter decrease at 4 weeks. The natural ability of the body to infection was observed in this study. There was also a decrease in MDA and  $H_2O_2$  in groups pretreated with the extract. The ability of the extract to quench free radicals generated during *T. brucei* was demonstrated by its ability to reduce markers of oxidative stress and increase reduced glutathione (GSH) in the erythrocytes. The reduced glutathione (GSH) is an intracellular nonenzymatic antioxidant defence system in the body. This is related to a previous experiment which proved a significant decrease in MDA with treatment with *A. indica* in cisplatin-induced hepatotoxicity and oxidative stress in female rats.<sup>[56]</sup>

Chemopreventive potential of *A. indica* had previously been demonstrated, as *A. indica* increased the activities of antioxidant enzymes, such as glutathione S-transferase and GPX and completely inhibited chemically induced hepatocarcinogenesis in rats.<sup>[54]</sup> SOD, CAT and GPX are enzymatic antioxidant that mop-up free radicals and ROS. SOD is the first line of defense against oxidative stress. It dismutates superoxide anion to  $H_2O_2$  and water. The  $H_2O_2$  generated from the activity of SOD will be detoxified to water and oxygen by CAT and GPX. Hence, CAT and GPX are positioned in the second line of defence to scavenge free radical and ROS. This study showed that CAT, SOD, GPX activities were inhibited with *T. brucei* infection and a dose-dependent increase was observed

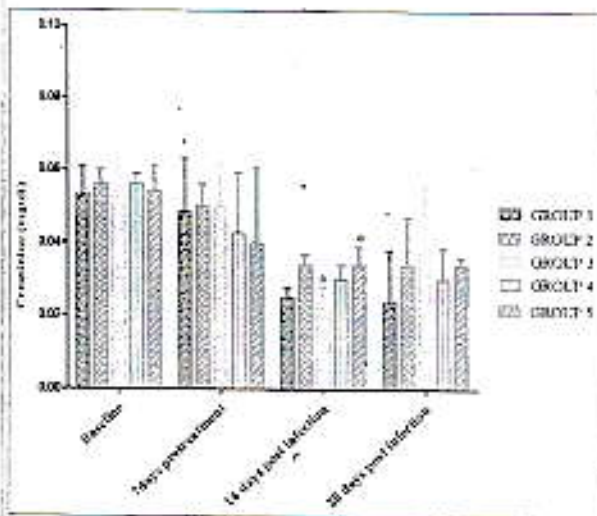


Figure 15: Effect of AI on Creatinine in experimental *T. brucei* infection. Values are presented as mean  $\pm$  standard deviation. Superscript (a) Indicates statistically significant when groups II, III, IV and V are compared with group I. Superscript (b) Indicates statistically significant when groups III, IV and V are compared with group II

with the highest at 200 mg/kg *A. indica*. The reduced glutathione significantly increased in dogs pretreated with *A. indica* (50 mg/kg and 200 mg/kg) after 2 weeks of *T. brucei* infection. Previous study with *T. brucei* infection in rats reported a decrease GSH, MDA, SOD and CAT activities in the serum, liver and kidney.<sup>[21]</sup> Similarly, *T. brucei* infection in rats has also been reported to cause increase ALT, AST activities and urea content in the serum, liver and kidney respectively.<sup>[30]</sup> This, therefore, confirm the antioxidant potential of *A. indica* on ROS.<sup>[31]</sup>

*Trypanosoma brucei* has varying effect on total protein as hypoproteinemia as well as hyperproteinemia.<sup>[32]</sup> This study showed a significant increase total protein in the erythrocyte of dogs pretreated with 200 mg/kg of *A. indica* after 4 weeks of *T. brucei* infection. However, total protein in serum decrease significantly following pretreatment with 100 mg/kg and 200 mg/kg of *A. indica* after 4 weeks of infection with *T. brucei* infection. The significant increase and decrease may be due to a wave of total protein during *T. brucei* infection which was consequently ameliorated by *A. indica*.

Aqueous *A. indica* extract (500 mg/kg, p.o.) has been reported to significantly reduce elevated levels of AST, ALT in paracetamol induced liver damage in rats.<sup>[33]</sup> ALT, AST and ALP are liver marker enzymes that are released into the blood following damage to the membrane of the hepatocytes. In this study, *T. brucei* infection caused elevated ALT, AST and ALP levels which is in agreement with the elevated liver enzymes as reported by Takečt and Fagbemi.<sup>[34]</sup> There was no significant difference in the values of ALT, AST and

ALP in all the groups pretreated with *A. indica* compared to the *T. brucei* infection. This indicates the hepatoprotective potential *A. indica* as previously reported.<sup>[35]</sup>

Urea is produced by the liver as waste product of protein digestion in the urea cycle and is excreted from the body by the kidney. BUN had been found to increase with *T. brucei* infection.<sup>[21]</sup> In our study, there was no significant difference in the values of BUN in all the groups pretreated with *A. indica* and infected with *T. brucei*. Creatinine produced in the liver is converted to creatinine which is removed from the blood chiefly by the kidneys. There was a significant increase in creatinine after 2 weeks of *T. brucei* infection. There was also a significant decrease in creatinine with pretreated with 50 mg/kg *A. indica* after 2 weeks of *T. brucei* infection. This indicates that *A. indica* could attenuate nephropathy in *T. brucei* infection and as such acted as a nephroprotective agent. This finding is supported by an experiment whereby cisplatin-induced nephrotoxicity in Wistar rats was attenuated by *A. indica*.<sup>[36]</sup>

Myceloperoxidase had been linked to both inflammation and oxidative stress whereby increase ROS may increase MPO release from neutrophils.<sup>[37]</sup> This study showed that MPO values in serum and erythrocyte were significantly increased with *T. brucei* infection. There was a significant decrease in MPO values in groups pretreated with 100 mg/kg and 200 mg/kg of *A. indica* at 2<sup>nd</sup> week of *T. brucei* infection and also a significant decrease in MPO groups pretreated with 50 mg/kg of *A. indica* at the 4<sup>th</sup> week of *T. brucei* infection. This indicates that MPO released by neutrophils during *T. brucei* infection potentiated oxidative stress was ameliorated by *A. indica*.

## CONCLUSION

These results suggest that *T. brucei* infection increased lipid peroxidation, generated free radicals, inhibited enzymatic and nonenzymatic antioxidant machinery and increased serum creatinine and inflammatory response. However, pretreatment with *A. indica* showed a protective effect against erythrocyte oxidative stress and markers of inflammation. Thus, MEAI ameliorated *T. brucei* induced oxidative stress and inflammation through its antioxidant and anti-inflammatory properties. Therefore, the leaves of *A. indica* could be employed for the treatment of *T. brucei* infection as an alternative source to orthodox medicine in developing countries that are ravaged by poverty.

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