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Effects of aqueous extract of fruit pulp of *Adansonia digitata* L. on the oxidative stress profile against *Trypanosoma brucei brucei* infection in albino rats

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Abstract

Background: Chemotherapy is the most widely used means of controlling trypanosomosis, however, effectiveness of the drugs available is limited by a number of factors. This study investigates the oxidative stress profile of aqueous extract of the fruit pulp of *Adansonia digitata* on some organs in rats infected with *Trypanosoma brucei brucei*.

Methods: Thirty-five male albino rats were divided into 7 groups of 5 rats each. Groups B, C, D, E, F and G were inoculated with 0.20 ml of suspension containing 10^6 *T. b. brucei*. Group A were neither infected nor treated. Group B were infected but not treated. At onset of parasitaemia, rats in group C were treated with diminazene aceturate at 3.5 mg/kg body weight once, while rats in group D were treated with vitamin C at 200 mg/kg body weight for 3 days consecutively. Rats in groups E, F and G were treated orally for 3 days with the aqueous extract of fruit pulp of *A. digitata* at a dosage of 40 mg/kg, 80 mg/kg and 160 mg/kg body weight respectively. Liver and kidney tissues of the rats were collected at necropsy (10 days PI) for oxidative stress analysis.

Results: There was a significant ($p < 0.05$) effect in the concentration levels of malondialdehyde, superoxide dismutase, glutathione peroxidase and catalase among the different groups treated with aqueous extract of fruit pulp of *A. digitata*.

Conclusion: The extract of *A. digitata* exert protective effects against tissue peroxidation in albino rats experimentally infected with *T. b. brucei*.

Keywords: Aqueous extract, *Adansonia digitata*, Albino rats, Oxidative stress, *Trypanosoma brucei brucei*

Introduction

African animal trypanosomosis affects most domestic animals and is caused by blood dwelling protozoan parasites of the genus *Trypanosoma*. Trypanosomosis is considered the most important livestock disease after Contagious bovine pleuropneumonia (CBPP) and remains a major obstacle to livestock production in Nigeria [1, 2]. Trypanosomes are microscopic unicellular protozoa found in the blood of man and some domestic and wild animals. They are also present in the saliva and faeces of insect vectors [3–5]. Transmission of *Trypanosoma brucei* between mammalian hosts is usually by an insect vector, the tsetse fly. The parasite undergoes complex morphological changes as they move between insect and mammalian hosts over the course of their variant surface glycoprotein (VSG) coat, which undergoes remarkable antigenic variation, enabling persistent evasion of host adaptive immunity and thus chronic infection [6].

Trypanosoma brucei species is the causative agent of Human African Trypanosomosis (HAT) which is also known as sleeping sickness in man and Nagana or Animal African Trypanosomosis (AAT) in animals [7]. *Trypanosoma brucei* has traditionally been grouped into three subspecies; *Trypanosoma brucei brucei, Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense. Trypanosoma b.*

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gambiense and *T. b. rhodesiense* are mainly parasites of man, with *T. b. brucei* been the species that affects animals and on very rare occasions affects man [7, 8].

The control of animal trypanosomosis over the years, relied mainly upon two broad strategies; first is the use of chemotherapeutic agents to treat the infected animals and the second strategy is to control the vector [9]. In general, the chemotherapeutic approach is used more widely than vector control since it is easier to kill the trypanosomes than to destroy the vector [10]. Currently the use of chemotherapy for the treatment of African trypanosomosis is unsatisfactory due to the raising of the parasite resistance to the existing drugs, treatment failures and the high level of toxicity it causes to the infected animals [11]. Attention is now focused on the search for non-toxic and readily available natural products for the treatment of trypanosomosis, where many African medicinal plants such as *Morinda morindiodes*, *Tithonia diversifolia*, *Acalypha wilkesiana*, *Strophanthus sarmentosus*, *Carissa Spinarum*, *Butyrospermum paradoxum*, *Waltheria indica*, *Vernonia amygdalina*, *Morinda lucida* and *Hymenocardia acida* have been found to have anti-trypanosomal activities [12, 13].

Adansonia digitata which is also known as Baobab is a large iconic tree indigenous to Africa and many other countries. It is considered as an emblematic, culturally important and physically majestic sub-tropical tree. In the past decade, it has attracted the interest of several pharmaceutical companies and researchers due to its various traditional uses, such as medicinal, nutritional, and cosmetic [14]. Various parts of the plant (e.g. leaf, bark, fruit pulp), have traditionally been used as immune-stimulant, anti-inflammatory, analgesic, insect repellent and pesticide [15]. *A. digitata* is being used in the treatment of diarrhea and dysentery in many African countries and has been confirmed as a substitute for imported western drugs [15, 16]. The high natural content of vitamin C in Baobab fruit pulp is well-documented for its antioxidant capability [17], which may be used in the prevention of oxidative stress and the treatment of related disease conditions [18].

The impact of trypanosomosis on animal and human health and the economy is enormous, thereby necessitating continuous research for better ways of eradicating the disease [19]. In order to tackle the lingering problems associated with the chemotherapy of African trypanosomosis, in recent years, scientists have focused their attention towards the search for effective ethno-botanical treatment for the disease [12, 19]. Consequently, many African medicinal plants were discovered to have some anti-trypanosomal activities [12] including *A. digitata* [20], and to the best of our knowledge there are no reports on the oxidative effect of the extract of fruit pulp of *A. digitata* following trypanosome infection. This study investigates the effects of aqueous extract of fruit pulp of *A. digitata* on the in vivo antioxidant defense system of the liver and kidney of Albino rats infected with *T. b. brucei*.

Materials and methods

Plant material

The fruit of *A. digitata* were harvested from the tree at the back of the Department of Parasitology and Entomology, Ahmadu Bello University, Zaria. The fruit pulp was harvested dried from the tree and the pod was broken and the pulp was removed from the seed and stored until needed.

Preparation of aqueous extract of *A. digitata*

Two hundred and eighty grams of the dried pulp of *A. digitata* was placed in a conical flask and seven liters of distilled water was added and left to stand for 72 h. The mixture was then filtered using 850 nm and 150 nm pore size sieves, respectively. The third stage of filtration was done using Whatman filter paper no.1 and cotton wool was put in the filter paper to get a pure solution. It was then frozen and dried using freeze-drying machine.

Preliminary phytochemical screening

Phytochemical analysis of the fruit pulp of *A. digitata* was performed according to the methods described by Sofowora [21] and Evans [22].

Ferric chloride test was used to test for tannins. About 0.5 g of the dried aqueous extract was diluted with distilled water in a ratio of 1:4 and few drops of 10% ferric chloride solution were added. A blue or green colour indicated the presence of tannins.

To test for saponins, about 0.5 g of the dried aqueous extract was boiled. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml of distilled water in a test tube. The test tube was corked and shaken vigorously for about 30 s, and then it was allowed to stand for half an hour. A honey comb forth was an indicator of the presence of saponins.

Carbohydrates was tested by adding a few drops of Molisch's reagent to 2 ml each of the water extract in two tubes. A small quantity of concentrated sulphuric acid was then added and allowed to form a lower layer. A purple ring at the interface of the liquids indicated the presence of carbohydrates. Each mixture was then shaken and allowed to stand for 2 min and diluted with 5 ml of water. A purple precipitate showed the presence of carbohydrates.

Glycosides was detected by adding Fehling's reagent to 0.1 g of the dried aqueous extract, and the mixture was boiled for 2 min. A brick red colouration indicated the presence of glycosides.

The Shinoda test was used to test for the presence of flavonoids. This was carried out by adding 0.5 g of the dried aqueous extract to four pieces of magnesium fillings,

Table 1 Experimental grouping of rats and the experimental regimen

Experimental groups	Experimental Regimen
A	Not infected with *Trypanosoma b. brucei* and not treated.
B	Infected with *T. b. brucei* and not treated
C	Infected with *T. b. brucei* and treated with diminazene aceturate at 3.5 mg/kg body weight intra peritoneally
D	Infected with *T. b. brucei* and treated with vitamin C at 200 mg/kg body weight orally for 3 days.
E	Infected with *T. b. brucei* and treated with 40 mg/kg body weight of the extract orally for 3 days
F	Infected with *T. b. brucei* and treated with 80 mg/kg body weight of the extract orally for 3 days
G	Infected with *T. b. brucei* and treated with 160 mg/kg body weight of the extract orally for 3 days.

followed by few drops of concentrated hydrochloric acid. A pink or red colour indicated the presence of flavonoids.

For alkaloids testing, five test tubes were used for the sample. Few drops of the following reagents: manager's reagent, Dragendorff's reagent, Mayer's reagent and 10% Picric acid solution were added respectively to each of the five test tubes. The presence of precipitate in at least 3 or all of the above reagents indicated the presence of alkaloids.

Quantitative phytochemical analysis

For the determination of saponins content, 2 g of fruit pulp extract was weighed into a 250 ml conical flask. 10 ml of 20% ethanol (C_2H_5OH) was added, the mixture was heated in a hot water bath for 4 h with continuous stirring at about 55 °C. The mixture was then filtered and the residue re-extracted with another 20 ml of 20% ethanol (C_2H_5OH). The combined extract was concentrated to 16 ml in a hot water bath at about 90 °C. The concentrated extract was then transferred into a 250 ml separating funnel and 20 ml of diethyl ether $(CH_3CH_2)_2O$ was added to the extract and shaken vigorously. The aqueous layer was recovered while the diethyl ether $(CH_3CH_2)_2O$ layer was discarded and the purification process was repeated. 60 ml of n-butanol (C_4H_9OH) was then added and the combined n-butanol was washed with 10 ml 5% Sodium Chloride (NaCl). The remaining solution was then heated in a hot water bath to evaporate to dryness and the residue was then weighed. The saponins content was calculated as percentage [23].

The total phenolic content of the extract was evaluated using the Folin–Ciocalteu method with modifications as documented by Baba and Malik [24].

To determine the content of flavonoids, 500 mg of the fruit pulp extract was repeatedly extracted with 10 ml of 8% aqueous methanol at room temperature. The mixture was then filtered using Whatman No 1 filter paper. The filtrate was transferred into a 250 ml beaker and was put in a hot water bath and allowed to evaporate to dryness. It was then weighed and the percentage was calculated as described by Krishnaiah et al. [25].

In determining the Alkaloids content, 200 mg of the fruit pulp extract was weighed into a 250 ml beaker and 8 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 h. It was filtered and the extract was concentrated in a hot water bath to one quarter of the original volume. Concentrated Ammonium hydroxide (NH_4OH) was then added drop wise to the extract until the precipitation was completed. It was washed with diluted Ammonium hydroxide and then filtered. The residue was the alkaloid, which was then dried and weighed. The percentage alkaloid was calculated using the formula of Kumar and Bhardwaj [26].

Inoculum

Trypanosoma b. brucei (Federe strain) was obtained from the Nigerian Institute for Trypanosomosis Research (NITR), Kaduna and passage into healthy rats to maintain the parasite continuously before the onset of the experiment. Collected blood was diluted in physiological saline before being used for the inoculation of the experimental animals.

Experimental animals

Laboratory bred albino male rats weighing 100–150 g each (8–12 weeks old) were purchased from the Animal House unit of NITR, Vom, Plateau State. The rats were housed in groups where each group contained 5 rats which were kept in clean cages, in a well-ventilated room. The rats

Table 2 Phytochemical Screening of aqueous extract of fruit pulp of *A. digitata* L.

No	Phytochemical constituents	Inference
1	Tannins	+
2	Saponins	+
3	Phenol	+
4	Terpenoid	+
5	Anthraquinone	+
6	Reducing sugar	+
7	Steroids	+
8	Glycosides	+
9	Flavonoids	+
10	Alkaloids	+

+ = Detected

Table 3 Quantitative phytochemical analysis of aqueous extract of fruit pulp of *A. digitata* L.

No	Phytochemical constituents	Inference
1	Saponins	0.10%
2	Total Phenol	16.14 mg/g
3	Flavonoids	3.51%
4	Alkaloids	0.07%

were fed with pelleted grower feed obtained from a commercial feed outlet (Vital Feeds Plc, Plateau State, Nigeria) and water was given ad libitum. The rats were allowed to acclimatize for 2 weeks before commencing the experiment.

Experimental grouping and infection

The 35 acclimatized adult male rats were divided into seven groups (A, B, C, D, E, F and G) of five rats each as shown in Table 1. Rats in group A were not infected with the parasite and served as the non-infected control group while rats in group B, C, D, E, F and G were inoculated with 0.20 ml of infected blood from the donor rat containing 10^6 *T. b. brucei*. At the onset of parasitaemia (6 days post infection) the infected rats were not treated/treated as shown in Table 1.

Sample collection

At the termination of the experiment, the rats were sacrificed and tissues from liver and kidney were collected and put in a bottle containing normal saline to analyze for Malondialdehyde (MDA) using method described by Fraga et al. [27]. The Superoxide dismutase (SOD) activity was measured based on its ability to inhibit the auto oxidation of epinephrine as described by Misra and Fridivisah [28]. The catalase activity was monitored as described by Aebi [29] and the reduced glutathione was determined using the DTNB method as described by Ellman [30]. The liver and kidney of albino rats were assessed for this study as these organs are markers indicating of injury/insult to the host [31].

Data analysis

Descriptive statistics was performed on the data. The mean values and the standard error of the mean was used in plotting the charts. One-way ANOVA with Turkey's multiple comparison test was performed using GraphPad Prism Version 4.00 for windows. A value of $p < 0.05$ was considered significant.

Results

The result of the phytochemical screening of the aqueous extract of fruit pulp of *A. digitata* is presented in Table 2. Ten different phytochemicals were detected.

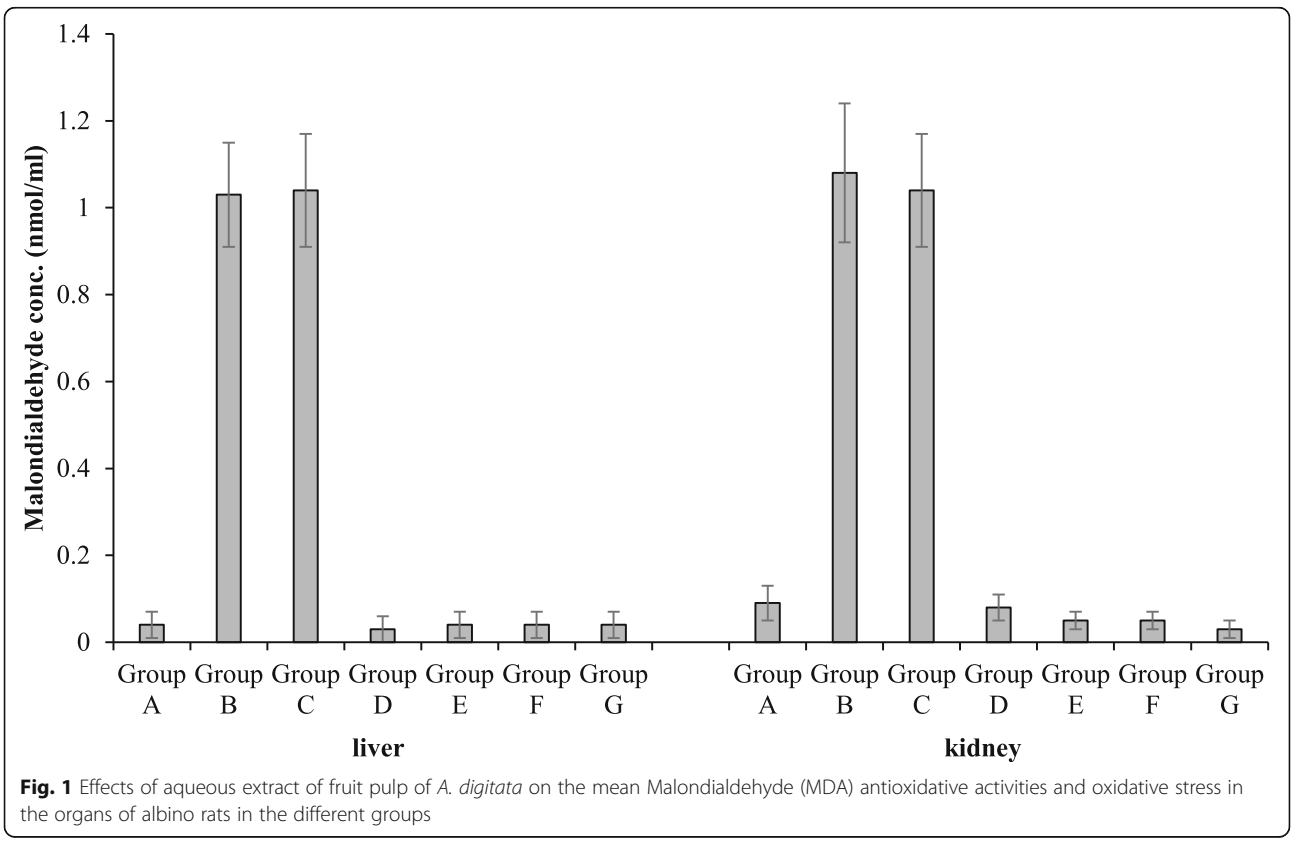

Fig. 1 Effects of aqueous extract of fruit pulp of *A. digitata* on the mean Malondialdehyde (MDA) antioxidative activities and oxidative stress in the organs of albino rats in the different groups

The quantitative phytochemical analysis shows that the fruit pulp of *A. digitata* L. aqueous extract is rich in Total phenol (16.14 mg/g), Flavonoids (3.51%), Saponins (0.10%) and Alkaloids (0.07) (Table 3).

There was a significant ($p < 0.05$) increase in the mean MDA level of the liver in groups B (1.03 mol/ml) and C (1.03 mol/ml) compared to other groups (0.03 mol/ml - 0.04 mol/ml), while that of the kidney recorded a similar trend with the mean MDA level being 1.08 mol/ml (group B), 1.04 mol/ml (group C) and that of groups A, D to G ranged between 0.03 mol/ml to 0.09 mol/ml (Fig. 1). The effect of the aqueous extract of fruit pulp of *A. digitata* on the mean SOD antioxidative activities in the liver and kidney of albino rats within the different groups is presented in Fig. 2. The mean level of SOD activity in the liver of groups B and C was 35.26 U/ml and 39.60 U/ml respectively, this was significantly lower compared to the other groups (the uninfected and untreated group, groups infected and treated with vitamin C, and 40 mg/kg, 80 mg/kg and 160 mg/kg respectively of the fruit pulp of *A. digitata*). The mean SOD level in the kidney was lowest in groups B (26.59 U/ml) and C (25.54 U/ml) compared to other groups (34.28 U/ml to 115.00 U/ml) and the difference was statistically significant ($p < 0.05$).

The mean Catalase activity in the liver was significantly higher ($p < 0.05$) in groups A (634.07 U/ml), D (311.24 U/ml), E (582.64 U/ml), F (378.11 U/ml) and G (597.43 U/ml) compared to that of groups B (147.53 U/ml) and C (186.48 U/ml), while that of the kidney showed a similar pattern (Fig. 3). Figure 4 shows the effect of the aqueous extract of fruit pulp of *A. digitata* on the mean Glutathione peroxidase antioxidative activities in the organs of albino rats within the different experimental groups. There was a significant increase in the liver activity of Glutathione peroxidase in groups A, D, E, F and G compared to that of group B and C, while the kidney activity of Glutathione peroxidase was significantly higher in groups F (37.67 u/g) and G (27.92 u/g) compared to that of the other groups that ranged between 16.00 u/g and 22.75 u/g.

Discussion

The diseases caused by *T. brucei* subgroups has been reported to cause hepatocellular degeneration and glomerulonephritis in addition to anemia [32], which is largely attributed to the large number of free radicals and superoxide generated by the trypanosomes that attacks membrane polyunsaturated fatty acids and proteins. This results to cellular injuries and

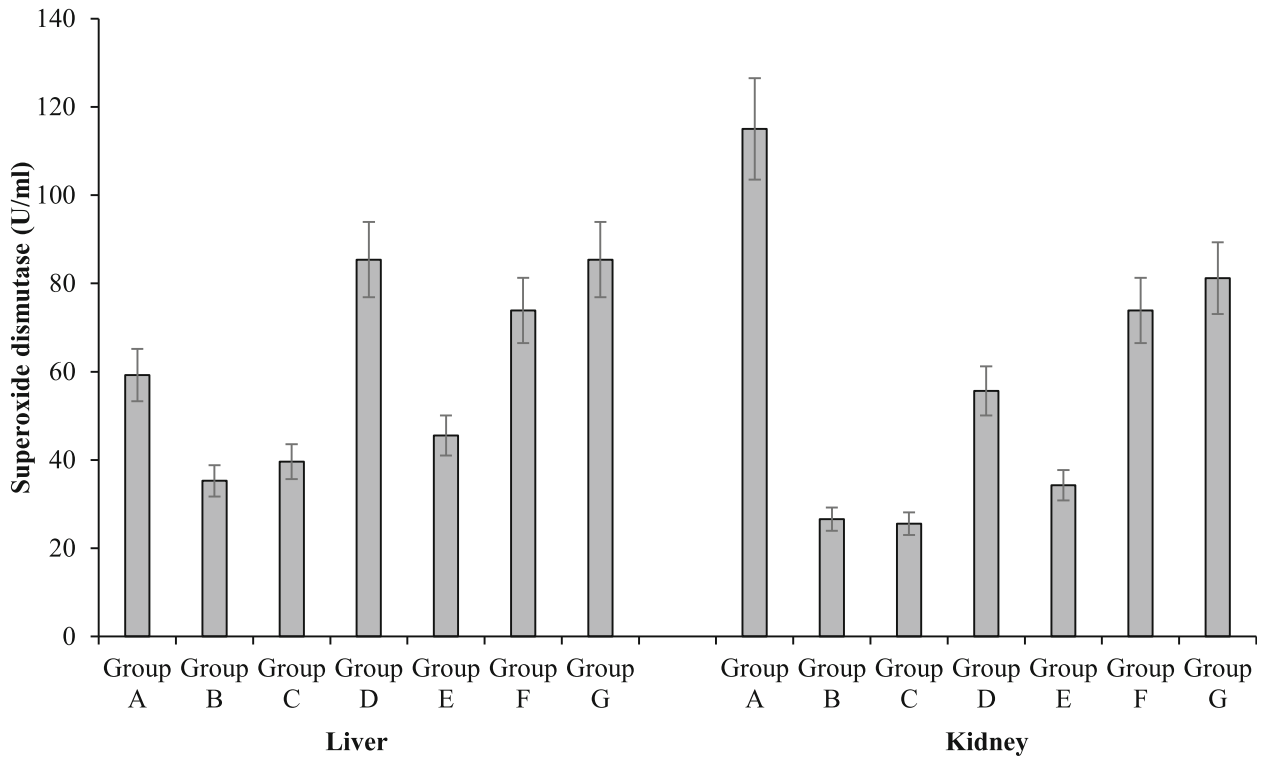

Fig. 2 Effects of aqueous extract of fruit pulp of *A. digitata* on the mean Superoxide dismutase (SOD) antioxidative activities and oxidative stress in the organs of albino rats in the different groups

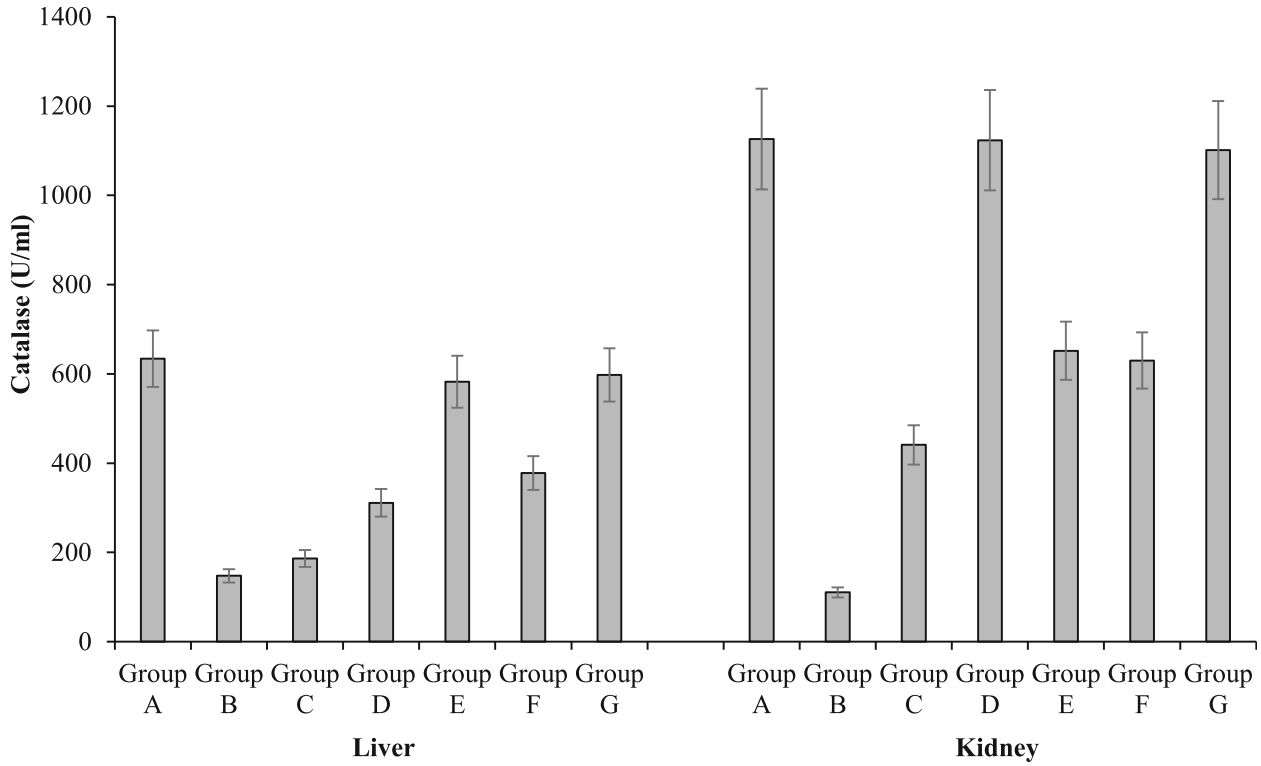

Fig. 3 Effects of aqueous extract of fruit pulp of *A. digitata* on the mean Catalase antioxidative activities and oxidative stress in the organs of albino rats in the different groups

consequently affecting vital tissues and organs of the infected hosts (animals and man) [33].

Tannins, saponin, phenol, glycosides, flavonoids and alkaloids which were part of the phytochemicals obtained in the screening of the aqueous extract of fruit pulp of *A. digitata*, are phytochemicals that exhibits physiological activities, with protective and disease preventing properties and as such possess promising medicinal potentials [34]. Phenols are ubiquitous secondary metabolites in plants and possess a wide range of therapeutic uses such as antioxidant, anticarcinogenic, antimutagenic, free radical scavenging activities and also decrease cardiovascular complications [35]. Flavonoids exhibit numerous biological effects such as anti-allergic, anti-cancer, anti-inflammatory, anti-hepatotoxic, anti-ulcer and antiviral activities. They are capable of effectively scavenging the reactive oxygen species (neutralizing the free radicals) because of their phenolic hydroxyl groups, thereby making them potent antioxidants and a potential candidate for the treatment of trypanosomiosis [35–37]. Alkaloids are chemical compounds mostly containing basic nitrogen atoms and are used as a remedy for gout with analgesic, muscle relaxant, antitumor, vasodilator, antihypertensive and anti-malarial activity [34, 38]. The presence of these classes of compounds especially flavonoids and alkaloids in the aqueous extract of the fruit pulp of *A. digitata* could be responsible for its various medicinal and antioxidative uses [39].

Malondialdehyde is a lipid peroxidation product that causes cellular damage by reacting with lipids, thereby causing peroxidation and release of products including hydrogen peroxide, [40]. An increase in the MDA level of the liver and kidney homogenate of rats infected with *T. b. brucei* and not treated and the group treated with diminazene aceturate suggests hepatic and kidney oxidative stress [40]. This stress was ameliorated in the groups treated with vitamin C and the extract of the fruit pulp of *A. digitata* by decreasing the activity of the enzyme. Similarly, ellagic acid, a phenolic acid found in plants and is produced from the hydrolysis of tannins, ameliorated the effect of *T. congolense*-induced liver and kidney damage by significantly reducing the level of MDA [41, 42].

Superoxide dismutase (SOD) is an enzyme that scavenge superoxide ion to either molecular oxygen or hydrogen peroxide, this superoxide is a free radical that causes oxidative damage in many cells [43], therefore the reduction in the SOD level in the liver and kidney of rats infected with *T. b. brucei* and not treated and those treated with diminazene aceturate suggests hepatic and kidney oxidative stress [43].

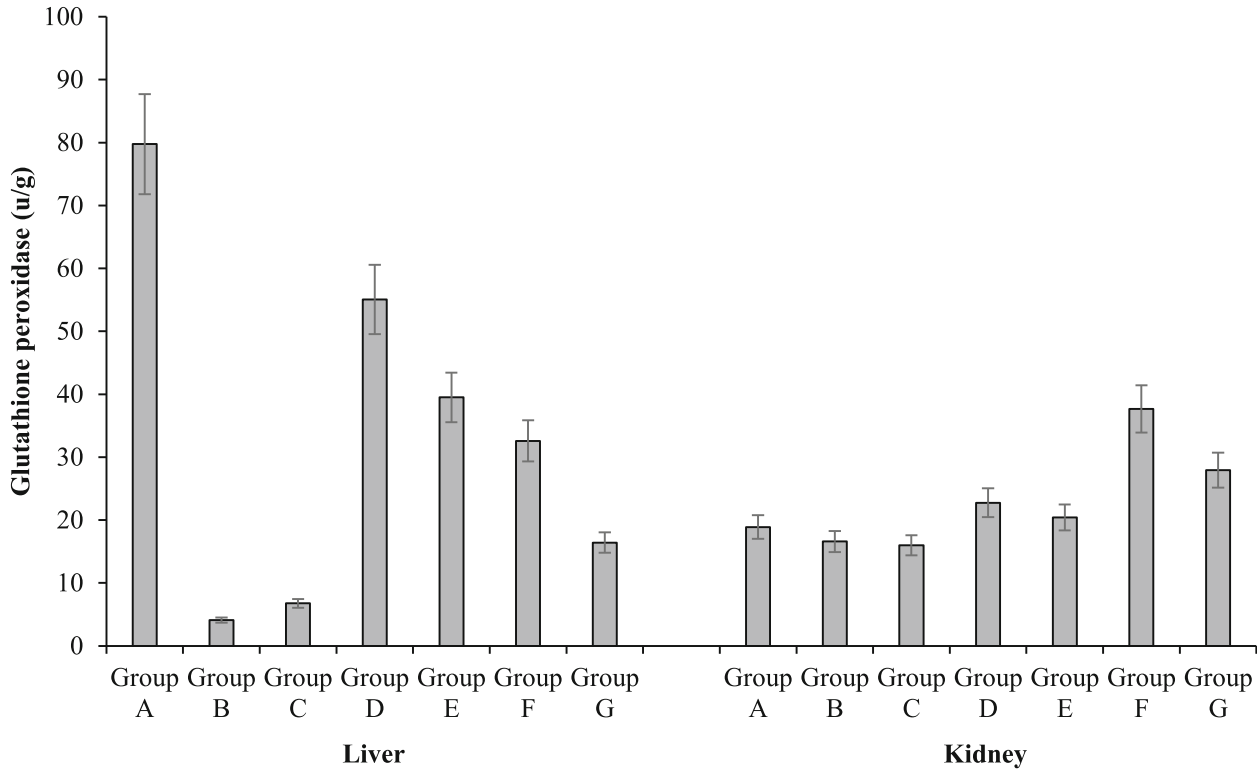

Fig. 4 Effects of aqueous extract of fruit pulp of *A. digitata* on the mean Glutathione peroxidase antioxidative activities and oxidative stress in the organs of albino rats in the different groups

However, this stress was ameliorated in the group treated with vitamin C, and those treated with 40 mg/kg, 80 mg/kg and 160 mg/kg body weight of the extract of fruit pulp of *A. digitata*. This is evidenced by the increase in the activity of this enzyme (SOD) in the liver and kidney tissues of rats treated with Vitamin C and the extract of the fruit pulp of *A. digitata*.

From this study, the reduction in catalase activity in the liver homogenate of the group infected with *T. b. brucei* and not treated and the group treated with diminazene aceturate showed hepatic and kidney oxidative stress. The extract of fruit pulp of *A. digitata* was able to reduce the oxidative stress in the treated groups by increasing the catalase activity. Catalase is an enzyme that catalyzes the conversion of hydrogen peroxide to water and molecular oxygen, and as a result, protects the body from oxidative abuses [44]. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species.

The non-enzymatic antioxidant system complements the activity of the enzymatic antioxidants system in the cells from excessive oxidative stress by acting as a reactive oxygen species (ROS) scavenger and modulating enzymes in vivo [45]. Reduced glutathione (GSH) is a natural antioxidant in all living organisms [46], the reduced glutathione (GSH) level in the rats infected with *T. b. brucei* and not treated and those treated with diminazene aceturate suggests hepatic and kidney oxidative stress condition, which indicates the exhaustion of the GSH in circulation, which leads to intracellular generation of reactive oxygen species. The groups treated with the plant extract showed an increase in the concentration of glutathione, thus ameliorating the effect of oxidative stress caused by the *Trypanosoma* species.

Conclusion

The extract of fruit pulp of *A. digitata* at dose range of 40 mg/kg to 160 mg/kg has a protective effect against tissue peroxidation in the liver and kidney of albino rats infected with *T. b. brucei*. The presence of alkaloids and flavonoids in the aqueous extract of fruit pulp of *A. digitata* could probably be responsible for the antitrypanosomal and antioxidant effects of the plant extract against *T. b. brucei* infection. It is hereby proposed that the fruit pulp of *A. digitata* be considered as a candidate for the treatment of sleeping sickness in humans (Human African Trypanosomosis) and Nagana (Animal African Trypanosomosis) in animals.

Abbreviations

AAT: Animal African Trypanosomosis; *T. b. brucei*: *Trypanosoma brucei brucei*; DTNB: (5–5′-dithiobis [2-nitrobenzoic acid]); GSH: Glutathione;

MDA: Malondialdehyde; NITR: Nigerian Institute for Trypanosomosis Research; ROS: Reactive oxygen species; SOD: Superoxide dismutase

Authors' contributions
OOO conceived, designed and carried out the laboratory experiments, wrote the manuscript; IDJ designed and supervised the study; AJN designed and co-supervised the work; SDO read, improved and prepared the manuscript for publication. The authors read and approved the final manuscript.

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Availability of data and materials
Research data and materials can be provided on request.

Ethics approval
Approval was obtained from the ethics committee of the faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. All applicable international, national, and/or institutional guidelines for the care and use of animals were adequately followed.

Consent for publication
Not applicable.

Competing interests
There is no conflict of interest in this study. Also, all the authors have declared that they have no conflict of interest.

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