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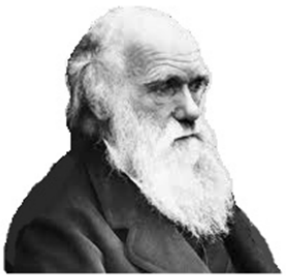

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Aqueous Extracts of Ginger (*Zingiber officinale* Roscoe) and Garlic (*Allium satium* L.) Bulbs: Phytochemical Screening and *In vivo* Antitrypanosomal Effect

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ABSTRACT

Aims: To evaluate the phytoconstituent and antitrypanosomal effects of aqueous extracts of Ginger and Garlic bulbs in mice experimentally infected with *Trypanosoma brucei brucei.* Design: A total of 30 adult male mice (weighing 25-40 g) were randomly grouped into six groups (I, II, III, IV, V, and VI) of 5 animals each. Five Groups (II-VI) were intraperitoneally injected with *T. brucei brucei* (5×10^5 cells/ml). Methods and Material: Aqueous extract of ginger and garlic bulbs were obtained using the procedure described by Wabo Pone and the extracts were subjected to phytochemical screening using the standard screening method of Silva. Also, each mice was inoculated with 0.1 ml of blood containing approximately 5×10^5 cells/ml as described by Herbert and Lumsden. Finally, the aqueous extracts were tested on the inoculated mice. Statistical analysis used: Data obtained were expressed as mean ± standard error of the mean (SEM), and subjected to one-way analysis of variance (ANOVA), SPSS 17.0 statistical software. $p<0.05$ was considered significant. Results: The aqueous extracts increased the survival time, packed cell volume, rectal temperature, and body weight (Ginger extract

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only) of mice infected with *Trypanosoma brucei brucei*. Phytochemical analysis revealed alkaloids, steroids, cardiac glycosides, phenol, and saponins in both Ginger and Garlic. Conclusions: Aqueous extracts of Ginger and Garlic bulbs do not have an anti-trypanosomal effect on *Trypanosoma brucei brucei*. Consequently, geographical location and time of collection of plants are factors that accept therapeutic on ginger and garlic on tryps and should be considered when testing the plants' efficacy on *Trypanosoma brucei brucei*.

***Keywords*:** Ginger, Garlic, Parasitaemia, Phytochemical screening, *Trypanosoma brucei brucei*

1. INTRODUCTION

Animal African Trypanosomosis (AAT) has continued to be a major restraint to cattle and other domestic animals in terms of their health and productivity in tsetse fly and other biting insects infested areas in some part of tropical Africa (Essan *et al.*, 2009). An estimated cost between $600 million and $1.2 billion yearly was spent in an attempt to control trypanosomosis in Africa (Olukunle *et al.*, 2010). Diminazene diacetate (DA) and isometamidium chloride hydrochloride (ISM) are the most presently used trypanocides for curative and prophylactic purposes respectively for control of this disease in cattle (WHO, 1995). Over the years, resistance to these drugs has been developed by these parasites (Anene *et al.*, 2006; Geerts *et al.*, 2001) which makes the exploration for efficacious chemotherapeutic agents from locally available ethnomedicinal plants for use as trypanocidal agents essential (Olukunle *et al.*, 2010). The dominant treatments of trypanosomosis are defied with problems comprising toxicity, drug resistance, and expensive/ limited drugs (Atawodi *et al.*, 2002).

Plants have been used for centuries in the ethnopharmacological treatment of dissimilar types of diseases and still offer the potentials for finding unique chemotherapeutic agents (Tagboto and Townson, 2001). Plants have a variety of bioactive compounds with activities against parasites, cancer, and viruses (Ahmed *et al.*, 2001). These plants comprise of compounds mainly secondary metabolites such as glycosides, alkaloids, coumarins, terpenes, and flavonoids (Rates, 2001). They have been reported to provide healthier and low-cost alternatives to synthetic chemotherapeutics (Adewummi *et al.*, 2001; Nok, 2005). In Nigeria, several ethnobotanical studies of Nigerian plants like *Khaya senegalensis*, *Moringa oleifera*, *Zingiber officinale*, and *Allium satium*, etc. used in the traditional management of trypanosomosis designated both important *in vitro* and *in vivo* antitrypanosomal activities.

Ginger (*Zingiber officinale*) is a perennial plant with bright green, narrow, yellowish-green flowers with purple markings and grass-like leaves. Ginger is cultivated in the tropics for its edible rhizome which is used for a range of purposes, including medicinal and cookery (Grant and Lutz, 2000). The efficacy of ginger is ostensible to be as a result of its carminative, absorbent, and aromatic properties (Govindarajan, 1982). *Allium sativum* is a perennial bulb with an erect, tall flowering stem that grows up to 2 and 3 feet. The plant yields purple to pink flowers that bud from July to September and the bulb is odiferous (McMahon and Vargas, 1993).

Increasing evidence demonstrates that oxidative stress plays an imperative etiologic role in the pathogenesis of African sleeping sickness (Edoga *et al.*, 2013; Ogunsanmi *et al.*, 2001; Kobo *et al.*, 2014). Oxidant stress arises when there is a disparity between radical-generating and scavenging activities. It may thus cause an escalation in the formation of oxidative products

(Ajakaiye *et al.*, 2013; Saleh *et al.*, 2009; Umar *et al.*, 2014). An infection caused by *the Trypanosoma brucei* group of parasites has been revealed to modify the antioxidant defense of the host (Omer *et al.*, 2007; Umar *et al.*, 2010; Serem *et al.*, 2013). Ginger and Garlic being strong antioxidants will either prevent or alleviate the cohort of free radicals (Haniadka *et al.*, 2013; Daleya *et al.*, 2013), such as those generated by trypanosomes (Shaba *et al.*, 2011). Studies on the active ingredients responsible for the trypanocidal activities of these plants are scanty. As well, the dosage of the extracts of Ginger and Garlic that will overwhelm or remove trypanosomes in animals with limited or no damage to vital organs needs to be elucidated.

Garlic (*Allium sativum*) contains essential alkaloids such as allin, allicin, ajoene, and diallyl sulfide S-allyl cysteine exhibiting, antiparasitic, anti-inflammatory, antiseptic, antibacterial, and immunomodulatory properties (Adibmoradi*et al*, 2006; Khan *et al*, 2012a; Chirom, 2020; Reynald, 2019). Ginger (*Zingiber officinale*) also, contains active ingredients such are gengerdiol. Gingerol, and gingerdoine (Khan *et al*., 2012b; Raza *et al.*, 2016; Zia ur Rehman *et al.*, 2018). The medicinal properties ascribed to ginger include anti-antitrypanosomal (Kobo *et al.*, 2014), arthritic (Srivastava and Mustafa, 1989; Bliddal *et al*, 2000), anti-migraine (Mustafa and Srivastava, 1990; Cady *et al.*, 2005), anti-thrombotic (Thomson *et al*, 2002), hypolipidaemic (Thomson *et al.*, 2002; Bhandari *et al.*, 2005; Al-Amin *et al.*, 2006), and anti-inflammatory (Thomson *et al*, 2002; Pennan*t al*, 2003),.

This work is aimed at evaluating the antitrypanosomal effects of aqueous extracts of *Zingiber officinale* (Ginger) and *Allium satium* (Garlic) bulbs in mice experimentally infected with *Trypanosoma brucei brucei* and also to evaluate the phytoconstituent in each aqueous plant extracts.

2. MATERIALS AND METHODS

2. 1. Ethical approval

All experimental protocols were approved and conducted with strict adherence to guidelines of the Institutional Animal Care and Use Committee of National Veterinary Research Institute, Vom, Plateau State, Nigeria, which are under the Constitution of the Federal Republic of Nigeria, Criminal Code Act. Cap C38 LFN 2004, Animal Diseases (Control) Act. Cap A17 LFN, 2004 and the Veterinary Surgeon Act Cap V3 LFN 2004.

2. 2. Plant materials

Fresh ginger and garlic bulbs were collected from Jos, Plateau State, Nigeria. It was identified at the Federal College of Forestry, Jos, Plateau State, Nigeria and with the voucher specimen number FHJ23020 for garlic specimen and FHJ23120 for ginger deposited in Forestry Herbarium Jos.

2. 3. Preparation of crude extracts

Ginger and Garlic bulbs were peeled and sliced into pieces to enhance drying. They were air under a shade for two weeks. The dried bulbs were then pulverized into fine particles mechanically using mortar and pestle. Aqueous extract of the bulbs was obtained using the procedure described by Wabo Pone et al. (2013). 100 grams of dried bulb of ginger and garlic were macerated in 1.5 litres of distilled water. The mixture was stirred daily and 48 hrs later the solution was filtered using 850 um and 150 um sieve.

The filtrate was further filtered with Whatman No. 1 filter paper to obtain a clearer filtrate. 200ml of each filtrate was distributed in four beakers, which were placed inside an oven heated at 50 °C. After one week, an aqueous extract of Ginger and Garlic was obtained and stored at +4 °C.

2. 4. Phytochemical screening

The aqueous extract of Ginger and Garlic were subjected to phytochemical screening in Toxicology Section, Biochemistry Division, National Veterinary Research Institute, Vom Plateau State, Nigeria, employing the standard screening method of Silva et al. (1998).

2. 5. Experimental animals

A total of 30 adult male mice weighing between 25 and 40 g were used for this experiment. They were obtained from the Animal House, National Veterinary Research Institute, Vom, Plateau State, Nigeria. The mice were acclimatized for 14 days in the research laboratory of Parasitology Division, Nigerian Institute for Trypanosomiasis Research, Vom, Plateau State, Nigeria, where the experiment was conducted. The mice were housed under standard hygienic conditions in plastic cages and kept at the ambient temperature of 24-26 °C with 12 h/day light period. They were given access to mice pellets and water ad libitum. CIOMS guidelines (1985).

2. 6. Trypanosome parasite

Trypanosoma brucei brucei used for this study was obtained from Nigerian Institute for Trypanosomiasis Research, Vom, Plateau State, Nigeria. The parasite was maintained by serial passages in donor mice and the parasitemia was monitored daily by preparing a wet mount and viewed under a light microscope (Olympus® CH23, Germany) at ×400 magnification.

2. 7. Inoculation of mice

The infected blood from a donor mouse was collected at peak parasitemia and diluted with physiological saline, which was inoculated into the peritoneal cavity of the infected mice. Each mouse was inoculated with 0.1 ml of blood containing approximately $5x10^5$ cells/ml (Herbert and Lumsden, 1976).

2. 8. Experimental design

A total of 30 adult male mice (weighing 25-40 g) were randomly grouped into six groups (I, II, III, IV, V, and VI) of 5 animals each. Five Groups (II-VI) were intraperitoneally injected with T. brucei brucei (5×10^5 cells/ml) and Group I was not infected as such; it served as uninfected control. Group II was infected and untreated to serve as untreated control, Group III was infected and treated with Diminazine Aceturate (3.5 mg/kg) intraperitoneally to serve as treated control, Groups IV was infected and treated with the synergy of aqueous Ginger and Garlic extracts in the ratio of 50:50, Group V was infected and treated with aqueous Garlic extract only, Group VI was infected and treated with aqueous Ginger extract only.

All these treatment groups (IV, V, and VI) received the extracts orally at a daily dose of 500 mg/kg body weight respectively for 5 days. The dose was chosen based on the acute toxicity (LD_{50}) done by Kobo et al. (2014) and Mikail et al. (2010).

2. 9. Rectal temperature

Rectal temperature was taken by inserting a digital thermometer into the rectum of the animal and their temperature read off.

2. 9. 1. Bodyweight Determination and Parasitaemia Monitoring

Bodyweight was measured weekly using a digital weighing balance, while parasitemia was monitored using the rapid matching method of Herbert and Lumsden, (1976).

2. 9. 2. Statistical analysis

Data obtained were expressed as mean ± standard error of the mean (SEM), and subjected to one-way analysis of variance (ANOVA), SPSS 17.0 statistical software. $p<0.05$ was considered significant.

3. RESULTS

A) Phytochemical screening

The phytochemicals present in the aqueous extracts are shown in Table 1.

Table 1. Phytochemical analysis of powdered bulb of Ginger and Garlic.

Phytochemicals	Result (Ginger)	Result (Garlic)
Saponins	+	+
Tannins	-	-
Steroids	+	+
Cardiac glycoside	+	+
Alkaloids	+	+
Flavonoids	-	-
Anthraquinone	-	-

- = phytochemical not detected; + = phytochemical detected.

B) Effect of treatments on body weight (BW) changes in mice

The results on the bodyweight of *T. brucei* infected mice treated with ginger and garlic extracts are presented in Figure 1. There was no significant ($p>0.05$) difference in body weights of mice that were exclusively treated with ginger or garlic as well as in the mice treated with both extracts when compared to the bodyweight of the controls (Group I, II, and III). Although,

there was an insignificant increase ($p>0.05$) in BW of mice treated with ginger at week 2 relative to other treated groups but was not statistically strong enough to establish a difference.

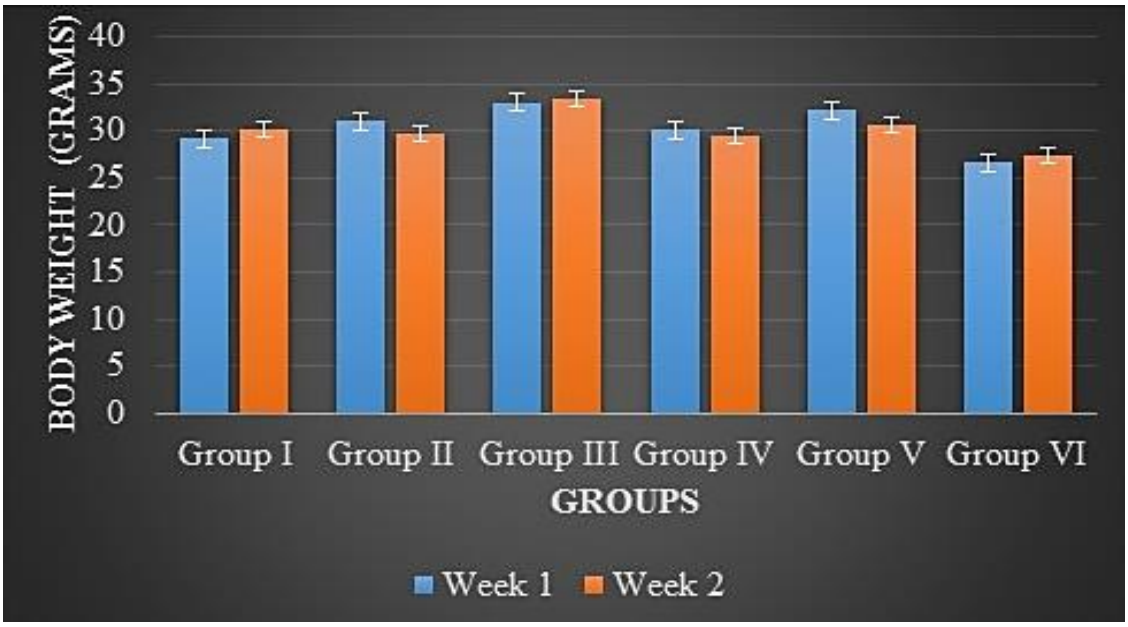

Figure 1. Effect of treatments with Diminazene Aceturate, ginger and garlic combined, Garlic alone, and Ginger alone extracts on the bodyweight of mice at week 1 and week 2

C) Effect of treatments on the survival rate (SR) of mice

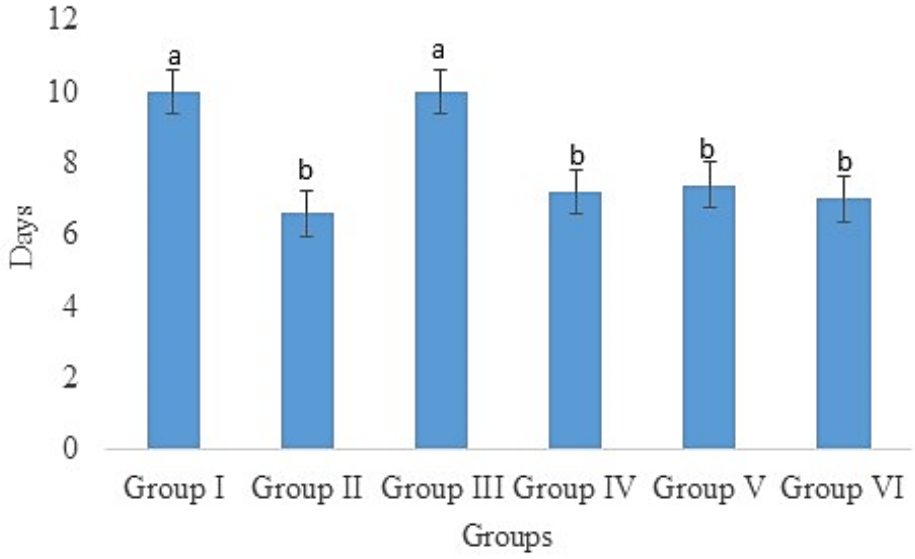

Figure 2. Effect of treatment with Diminazene Aceturate, ginger and garlic combined, Garlic alone, and Ginger alone extracts on the survival rate of mice experimentally infected with *Trypanosoma brucei brucei*.

The results on the survival rate of *T. brucei* infected mice treated with ginger and garlic extracts are presented in Figure 2. There was an insignificant ($p>0.05$) decrease in the SR of mice in Group II when compared to the treatment Groups (IV, V, and VI). Though, a significant ($p<0.05$) increase was observed in the SR of mice in Groups I and III when compared to treatment Groups (IV, V, and VI).

Values with the different alphabet superscripts (a, b) on different groups are significantly different; Values with the same alphabet superscripts are insignificantly different.

D) Effect of treatments on the level of parasitemia

A significant ($p<0.05$) decrease of the level of parasitemia in Group III mice, was observed at day 5 post-infection in comparison to Groups II, IV, V, and VI (Figure 3).

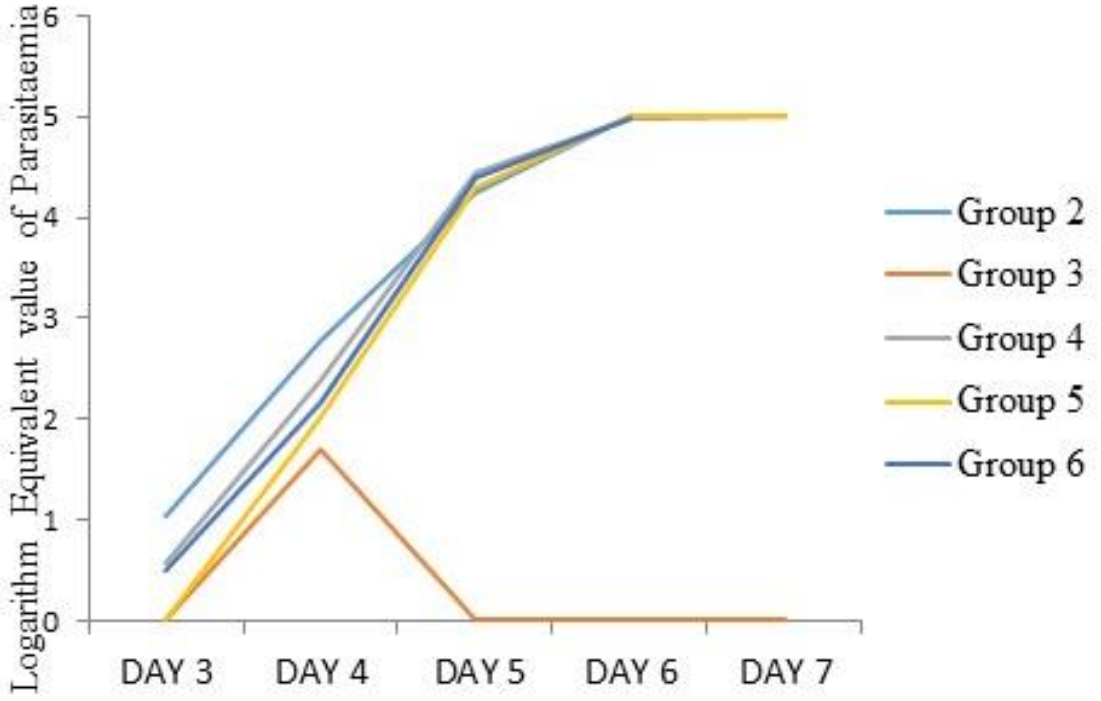

Figure 3. Effect of treatment with Diminazene Aceturate, ginger and garlic combined, Garlic alone, and Ginger alone extracts on the level of parasitemia in mice experimentally infected with *Trypanosoma brucei brucei*.

E) Effect of treatments on the packed cell volume (PCV)

The results on PCV of *T. brucei* infected mice treated with ginger and garlic extracts are presented in Figure 4. There was an insignificant ($p>0.05$) decrease in PCV of mice that were recorded in Group II in comparison to treatment Groups (IV, V, and VI) ranging from post-treatment to pre-patent. Although, there was a significant increase ($p<0.05$) in PCV, of mice in groups (I and III) at post-treatment relative to other treated groups but was not statistically strong enough to establish a difference.

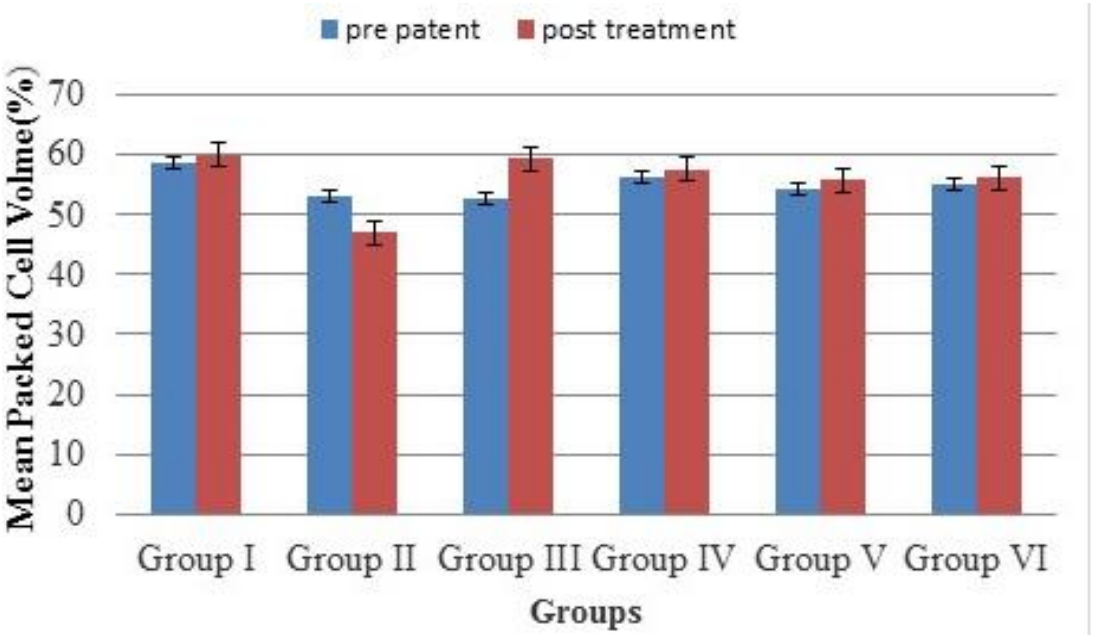

Figure 4. Effect of treatment with Diminazene Aceturate, ginger and garlic combined, Garlic alone, and Ginger alone extracts on the packed cell volume of mice at pre patent and post-treatment.

F) Effect of treatments on the Rectal Temperature (RT)

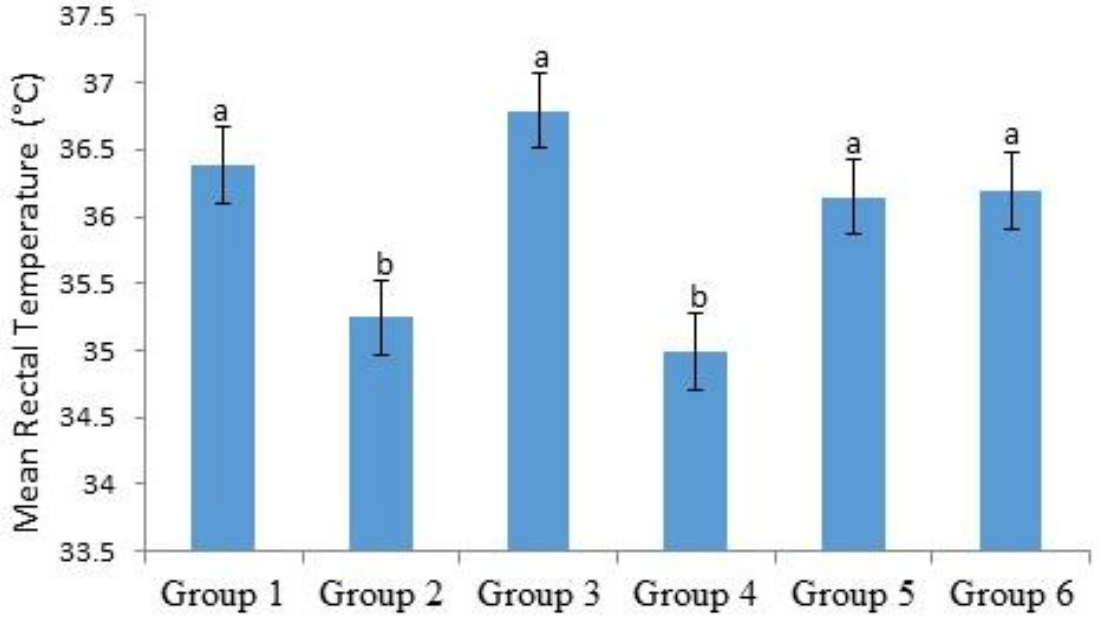

Figure 5. Effect of treatment with Diminazene Aceturate, ginger and garlic combined, Garlic alone, and Ginger alone extracts on the rectal temperature of mice experimentally infected with *Trypanosoma brucei brucei*

The effect of treatments on the rectal temperature of mice is shown in Figure 5. There was an insignificant ($p>0.05$) decrease in the RT of mice in Group II when compared to Groups V, and VI. Even though an insignificant ($p>0.05$) increase was observed in the RT of mice in Groups I and III when compared to Groups V and VI but the groups (I, III, V, VI) are significantly ($p<0.05$) different to groups (II and IV).

Values with the different alphabet superscripts (a, b) on different groups are significantly different; Values with the same alphabet superscripts are insignificantly different.

4. DISCUSSION

This study has shown that some phytochemicals such as alkaloids, saponins, steroids, and cardiac glycoside to be present in the aqueous extract of *Zingiber officinale* (ginger) and *Allium satium* (garlic) bulbs. Male mice (15) exposed to *Trypanosoma brucei brucei* were treated with a single dose (500 mg/kg) of combined ginger and garlic, garlic only, and ginger only which showed an increase in the survival time, packed cell volume, rectal temperature, and body weight (Ginger extract only). The findings from this study are related to a previous report on tryps exposure to plant extract; *Cucumis metuliferus* (Abubakar *et al.*, 2011) but in contrast to plant extracts; *Zingiber officinale* (Kobo *et al.*, 2014) and *Adansonia digitate* (Ogunleye *et al.*).

Potent bioactive substances in plants are abundant and varied in structural compositions (Chikezie et al., 2015). The presence of bioactive substances like alkaloids, saponins, steroids, and cardiac glycoside in the preliminary phytochemical screening of the bulbs of these plants declined the previously documentations on their phytochemical constituents (Kobo *et al.*, 2014; Mikail, 2010). It is vital to reference that phytochemical constituent analyses differ with the geographical location (Wadood *et al.*, 2013). Cardiac glycosides are used in the treatment of cardiac arrhythmias and congestive heart failure. These glycosides are found as secondary metabolites in numerous plants and some animals. Saponins were also found positive, soap nuts (Sapindus), especially *Sapindus mukorossi* is used medically as an emetic, expectorant, and for treatment of excessive epilepsy, salivation, migraines, and chlorosis. Sapindus is used in Ayurveda as a treatment of psoriasis, eczema, and for removing freckles.

The results on the bodyweight of *T. brucei* infected mice treated with ginger and garlic extracts are presented in Figure 1. There was no significant ($p>0.05$) difference in body weights of mice that were exclusively treated with ginger or garlic as well as in the mice treated with both extracts when compared to the bodyweight of the controls (Group I, II, and III). Although, there was an insignificant increase ($p>0.05$) in BW of mice treated with ginger at week 2 relative to other treated groups but was not statistically strong enough to establish a difference. This is in agreement with the findings of Alli *et al.* (2011) which showed that infection with *T. brucei brucei* was associated with bodyweight loss. The increase in body weight observed in Group III treated with Diminazine Aceturate (DA) and Group VI treated with ginger extract only (GI) demonstrated that DA and GI protected the mice from the trypanosome-induced decrease in body weight. The effect of DA and GI on body weight may be due to the trypanocidal effect of DA and the antioxidant effect of GI that may reduce the parasite burden in the body.

The relative increase (Figure 2) in the survival time of mice treated with combined ginger and garlic extracts, garlic extract only, and ginger extract only suggest that the extract could be useful in the management of Animal African Trypanosomosis. This finding approves with other

works that have shown that plant extracts having polyphenols have the ability to increase the survival rate of mice infected with *T. brucei* (Ngure *et al.*, 2009; Alli *et al.*, 2011). Trypanosomosis is a disease whose pathological effects are originated through the release of cytokines and nitric oxide (Serem *et al.*, 2013). Polyphenols have been shown to attenuate cytokine and nitric oxide-induced inflammation (Ngure *et al.*, 2009; Karina *et al.*, 2011), probably due to their reactive oxygen species (ROS) rummaging ability. Therefore, we may hypothesize that the ability of the extracts to increase the survival rate of mice in this study may be due to their ability to aid oxidative stress reduction and antioxidant defense system by protecting the defense system against the damaging effects of ROS. The survival time of mice treated with DA was significantly increased compared when compared to treatment Groups (IV, V, and VI). This can be due to the ability of the drug to remove the parasites from the blood (Saba *et al.*, 2007). The prepatent period of 2 days (Figure-3) observed in the present study disagrees with prior findings (Umar *et al*, 2007) that reported a prepatent period of 4-5 days in mice infected with *T. brucei*.

The parasitemia observed in this experiment rose steadily without any period of drop in all the treatment groups, which indicates an acute phase of the disease, except for the DA treated group where parasitemia dropped to 0 at 24 hours post-infection. In another study, a similar observation was made with this strain of parasite (Umar *et al.*, 1999). Treatment with combined ginger and garlic extracts, garlic extract only, and ginger extract only did not affect the onset of parasitemia and even at the end of the experiment when compared with untreated control (group II). Several researchers made similar observations on reduction in parasitemia and concluded that high parasite load could mask the crude extract efficacy (Ekanem *et al.*, 2008; Feyera *et al.*, 2014; Agbeyangi, 2017).

This could be responsible for the significant ($p<0.05$) increase in the level of parasitemia that was observed in the treated groups. More so, the efficacy of crude extracts may also require administration via the parenteral route. Reduced efficacy of crude extract of ginger and garlic extracts could also be due to enzymatic inactivation of active compounds or impaired absorption from the gut or both (Ekanem *et al*, 208; Feyera *et al*, 2014). Similarly, the geographical location where the plants are located and the time of collection of the plants may affect the efficacy of the plants.

The effect of treatments on the packed cell volume (PCV) changes of mice observed at prepatent period and post-treatment of the experiment (figure 4). Showed an insignificant ($p>0.05$) decrease on PCV of mice that were recorded in Group II in comparison to treatment Groups (IV, V, and VI) ranging from post-treatment to pre-patent. Although, there was a significant increase ($p<0.05$) in PCV, of mice in groups (I and III) at post-treatment relative to other treated groups but was not statistically strong enough to establish a difference.

It can be deduced that combined ginger and garlic extracts, garlic extract only, and ginger extract only can increase the PCV when compare to the untreated group which shows their little trypanosides potential against *T. brucei*. Although the increment of the treatment groups is not significant to the group with DA. Since *T. brucei* infection has been related to colossal production of hydrogen peroxide (Meshnick *et al.*, 1977), which resulted to increase the exposure of erythrocyte toward *in vitro* peroxidation.

The enhancement of PCV of the treated groups could therefore be due to the antioxidant effect of the crude extracts or their ability to reduce the free fatty acid levels which are reported to lyse red cells if they are not bound to albumin (Akanji, 1985). The cells are therefore regularly under attack from reactive oxygen species (ROS) or free radicals.

The effect of treatments on the Rectal temperature of mice was also examined (Figure-5). There was an insignificant ($p>0.05$) decrease in the RT of mice in Group II when compared to Groups V, and VI. Even though an insignificant ($p>0.05$) increase was observed in the RT of mice in Groups I and III when compared to Groups V and VI but the groups (I, III, V, VI) are significantly ($p<0.05$) different to groups (II and IV). As changes in body temperature is one of the symptoms of AAT which was measure through rectal temperature. Mice in group V and VI showed their trypanosides potential as they increase the rectal temperature that was reduced by *T. brucei*. But mice treated with DA as a standard trypanocidal increased the rectal temperature to normal more than the treatment groups.

The mechanism of the extracts' trypanocidal action was not determined. However, Sepulveda-Boza *et al.*, (1996) reported that many natural products exhibit their trypanocidal activity by their interference with the redox balance of the parasites acting either on the respiratory chain or on the cellular defenses against oxidative stress.

This is because natural products possess structures capable of generating radicals that may cause peroxidative damage to enzymes that are very sensitive to alteration in redox balance. It is also known that some agents also act by binding with the kinetoplast DNA of trypanosomes (Atawodi *et al*, 2003).

5. CONCLUSION

This study has shown that the aqueous extracts of *Zingiber officinale* (Ginger) and *Allium satium* (Garlic) bulbs contains phytoconstituents such alkaloids, steroids, Cardiac glycosides, phenol and Saponins that were previously detected in plants with anti-trypanosomal effects but do not contain some compounds like flavonoids and tannins which make the extracts to not have direct curative effects on *Trypanosoma brucei brucei* as there was no effect on parasitemia. Consequently, geographical location and time of collection of plants should be considered when testing the efficacy of plants on *Trypanosoma brucei brucei*.

However, the aqueous extracts increased the survival time, packed cell volume, rectal temperature and body weight (Ginger extract only) of mice infected with *Trypanosoma brucei brucei*.

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