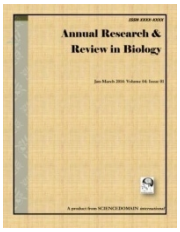

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In vivo Trypanocidal Activity of Ethanolic Crude Extract and Phytochemical Fractions of *Garcinia kola* Seeds

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Authors' contributions

The first author TOJ carried out all the laboratory analysis of the research and wrote the first draft of this article. The second author, BPO assisted with statistical analysis and production of the first draft.

Research Article

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ABSTRACT

Aim: To determine the trypanocidal activity of the crude ethanolic extract and phytochemical fractions of Garcinia kola seeds in *Trypanosoma brucei brucei* infected rats.
Study Design: *Trypanosoma brucei brucei* infected rats were treated with the ethanolic crude extract, alkaloid, flavonoid, saponin and cardiac glycoside fractions of *Garcinia kola* seeds. Parasitaemia and Packed cell volume (PCV) were measured.
Place and Duration of Study: Department of Biochemistry, University of Jos, Jos, Nigeria between June and October 2011.
Methodology: 11 groups of four rats each were used. Group A rats were infected but not treated. Groups B, C, D, E and F were infected but treated with 50mg/kg crude extract, alkaloid, flavonoid, saponin and cardiac glycoside fractions respectively, while groups G, H, I, J and K were infected but treated with 100mg/kg of the extracts respectively. Parasitaemia levels of rats were observed daily under Light microscope at x100 magnification. PCV values were determined on days 4 and 8 using microhaematocrit centrifuge at 10,000xg for 5 min.
Results: At 50mg/kg the crude extract brought about a 37.50% reduction in the parasitaemia of infected rats, the alkaloid fraction 76.70%, flavonoid fraction 53.45% and saponin fraction 76.70%. At 100mg/kg the crude extract and the alkaloid, flavonoid and

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saponin fractions reduced parasitaemia of infected rats by 84.50%, 92.25%, 61.20% and 68.98% respectively. Treatment with the cardiac glycoside fraction showed no improvement in parasitaemia. Significant increases (*P*=.05) in PCV values were observed in all the treated groups on days 4 and 8 when compared with the untreated ones.
Conclusion: Available results from this research shows that ethanolic crude extract of *Garcinia kola* seeds and its alkaloid, flavonoid and saponin fractions at 50 and 100mg/kg demonstrated activities against *Trypanosoma brucei brucei* in rats.

Keywords: In vivo; phytochemical; trypanocidal; Garcinia kola; parasitaemia.

1. INTRODUCTION

Human African Trypanosomiasis (HAT) and its livestock forms (Nagana and Surra) caused by protozoa of the genus Trypanosoma and transmitted by the tsetse fly are major threats to public health and economic developement in rural sub-Saharan Africa. HAT, which is one of the Neglected Tropical Diseases has received very little attention both locally and internationally [1]. Despite major advances in drug development in recent decades, essential medicines used in the treatment of HAT and other diseases that affects the world's poor are either too expensive, no longer produced, highly toxic or ineffective [2], hence the need to develop new treatments for these diseases.

Many natural products of plant origin have been reported to have activities against different species of protozoan parasites including *Plasmodium, Trypanosoma, leishmania* and *Entamoeba* [3]. *Garcinia kola* (*G. kola*) a species of flowering plant of the *Clusiaceae* or *Guttiferae* family found in the tropical rain forest region of West Africa is a plant known for its medicinal properties. The presence of bioactive compounds with high therapeutic properties in the seeds of the plant has been reported [4–11]. Phytochemical compounds that have been isolated from *G. kola* include oleoresin [12], tannins, saponins, alkaloids, cardiac glycosides [13]. Two new chromanols, garcioic acid and garcinal, together with σ - tocotrienol were also reported to be isolated from *G. kola* [14]. Other phytochemical compounds so far isolated from *G. kola* seeds are biflavonoids such as kolaflavone and 2-hydroxybi-flavonols [15,16,10]. Studies have shown that kolaviron, a natural biflavonoid from *G. kola* seeds possess the ability to protect against oxidation of lipoprotein in rats [17]. This activity which was demonstrated to be presumably by Fe^{2+} chelation and anti-oxidant activity might be of immense benefit in the management of African trypanosomiasis. It has been suggested that removal of excess iron through chelation could possibly prevent iron mediated injury to cells thereby reducing the pathology of anaemia and tissue damage associated with African trypanosomiasis [18].

This study is subsequent to our previous study on the *in vitro* trypanocidal activities of various phytochemical fractions obtained from *G. kola* seeds. The study showed that crude ethanolic extract of *G. kola* seeds and four phytochemical fractions obtained from it (alkaloid, flavonoid, saponin and cardiac glycoside fractions) exhibit varying degrees of trypanocidal activities *in vitro*. The alkaloid fraction showed the highest trypanocidal activity followed by the saponin fraction, the crude extract, the cardiac glycoside and the flavonoid fractions respectively [19]. In this study, the *in vivo* trypanocidal activity of the crude ethanolic extract and the alkaloid, flavonoid, saponin and cardiac glycoside fractions of *G. kola* seeds was determined.

2. METHODOLOGY

2.1 Extraction and Fractionation of *G. kola* Seeds

Ethanolic extraction of *G. kola* seed and subsequent fractionation were carried out using diverse organic solvents according to standard procedures [20]. The ethanolic crude extract was prepared using 100g of the powdered seed and 200ml of 70% ethanol. The extract was screened for the possible presence of phytochemicals such as Alkaloids, Tannins, Flavonoids, Saponins and Cardiac glycoside using the methods described in [21,22].

About 25.3g of dried ethanolic extract was redissolved in 250ml of distilled water and shaken vigorously. The suspension was filtered and the filtrate used for separation into various phytochemical fractions. Cardiac glycoside fraction was extracted with 5% KOH in n-butanol, saponin fraction with 10% HCl in n-butanol while alkaloid fraction was extracted in chloroform. Flavonoids were obtained from the remaining aqueous fraction [19,20]. The samples were appropriately dissolved in distilled water to obtain dosages corresponding to 50 and 100mg/kg rat weight.

2.2 Parasite

Bloodstream forms of *Trypanosoma brucei brucei* (*T. b. brucei*) was obtained from the Nigerian Institute of Trypanosomiasis Research, Vom, Nigeria.

2.3 Experimental rats

Wistar rats of an average weight of 150g were obtained from the animal house unit of the University of Jos. The rats were inoculated intraperitoneally with 0.1ml of innoculum containing about 10^3 trypanosomes/ml of infected blood in normal saline.

2.4 *In vivo* trypanocidal activity of the Crude Extract and Phytochemical Fractions of *G. kola* Seeds

The rats were divided into 11 groups of 4 rats each. Group A rats, which serve as the control group were infected but not treated. Groups B, C, D, E and F rats were infected but treated with 50mg/kg body weight of crude extract, alkaloid, flavonoid, saponin and cardiac glycoside fractions of *G. kola* seeds respectively. Groups G, H, I, J and K rats were infected but treated with 100mg/kg of the same extracts respectively. Oral administration of extracts to the infected rats which commenced on the first day parasites were sighted in the blood of rats was done twice daily until day 12 post infection. Parasitaemia levels of rats were observed daily under Light microscope at x100 magnification. Packed cell volume (PCV) was determined for each rat on days 4 and 8 using capillary tube on a microhaematocrit centrifuge at 10,000xg for 5 min and read on a microhaematocrit reader.

2.5 Statistical Analysis

Data obtained was subjected to analysis of variance and the significant differences between groups were determined using Duncan's Multiple Range test. Confidence interval was set at 95% level (P=0.05)

3. RESULTS

3.1 Phytochemical Screening of *G. kola* Seeds

Phytochemical screening of the crude ethanolic extract of *G. kola* seeds showed the presence of flavonoids, tannins, saponins, cardiac glycosides and alkaloids.

3.2 Effects of the Crude Extract and Phytochemical Fractions of *G. kola* Seeds on Parasitaemia Levels of Infected rats

The crude extract and the alkaloid, saponin, and flavonoid fractions of *G. kola* seeds at 50mg/kg and 100mg/kg body weight exhibited varying degrees of trypanocidal activities in the infected rats (Figs. 1–5). At 50mg/kg the crude extract brought about a 37.50% reduction in the parasitaemia of infected rats, the alkaloid fraction 76.70%, flavonoid fraction 53.45% and saponin fraction 76.70% (Fig. 6). At 100mg/kg the crude extract, alkaloid, flavonoid and saponin fractions reduced parasitaemia of infected rats by 84.50%, 92.25%, 61.20% and 68.98% respectively (Fig. 7). Infected rats treated with the cardiac glycoside fraction showed no improvement in parasitaemia just as the infected untreated ones. Both groups of rats did not survive beyond day 8 post infection at 50mg/kg and days 8 and 9 post infection respectively at 100mg/kg.

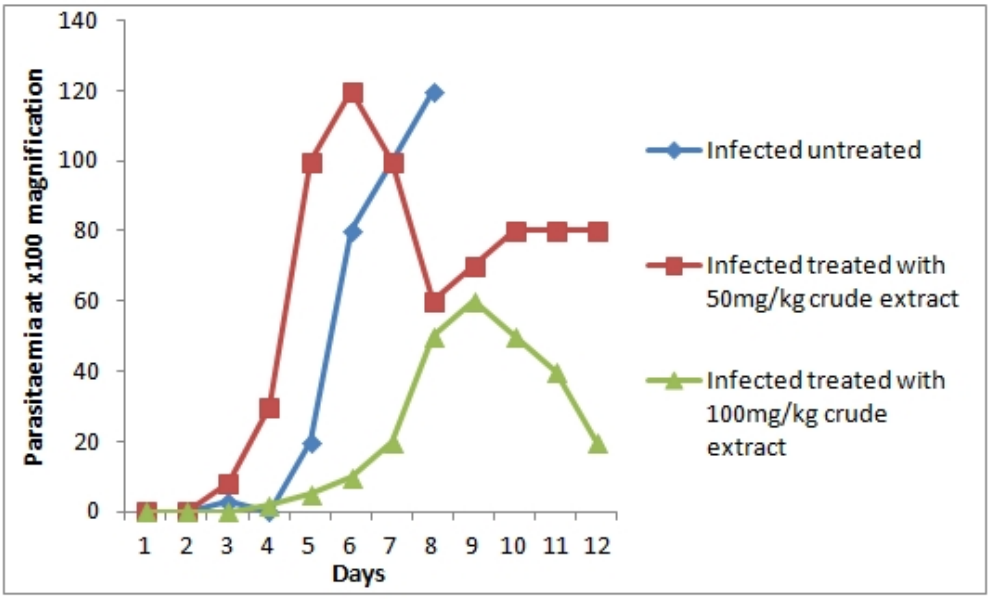

Fig. 1. Parasitaemia levels of *T. b. brucei* infected rats treated with 50mg/kg and 100mg/kg body weight of ethanolic crude extract of *G. kola* seeds. Plotted values are a representation of four determinations

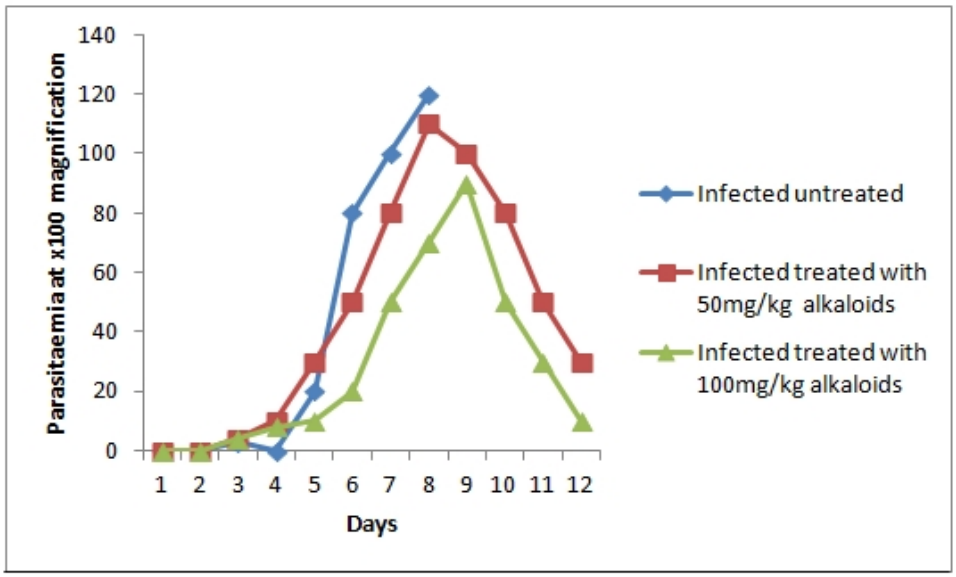

Fig. 2. Parasitaemia levels of *T. b. brucei* infected rats treated with 50mg/kg and 100mg/kg body weight of alkaloid fraction of *G. kola* seeds. Plotted values are a representation of four determinations

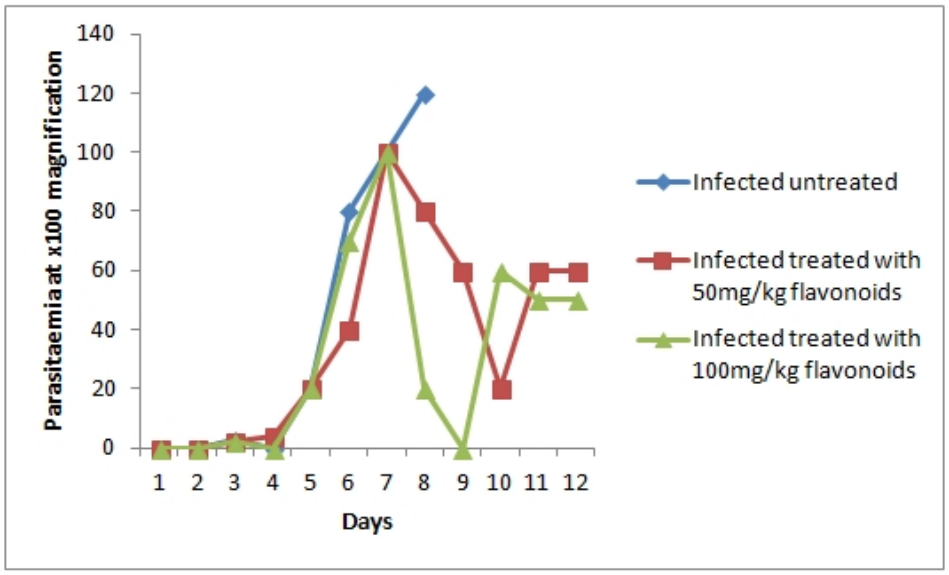

Fig. 3. Parasitaemia levels of *T. b. brucei* infected rats treated with 50mg/kg and 100mg/kg body weight of flavonoid fraction of *G. kola* seeds. Plotted values are a representation of four determinations

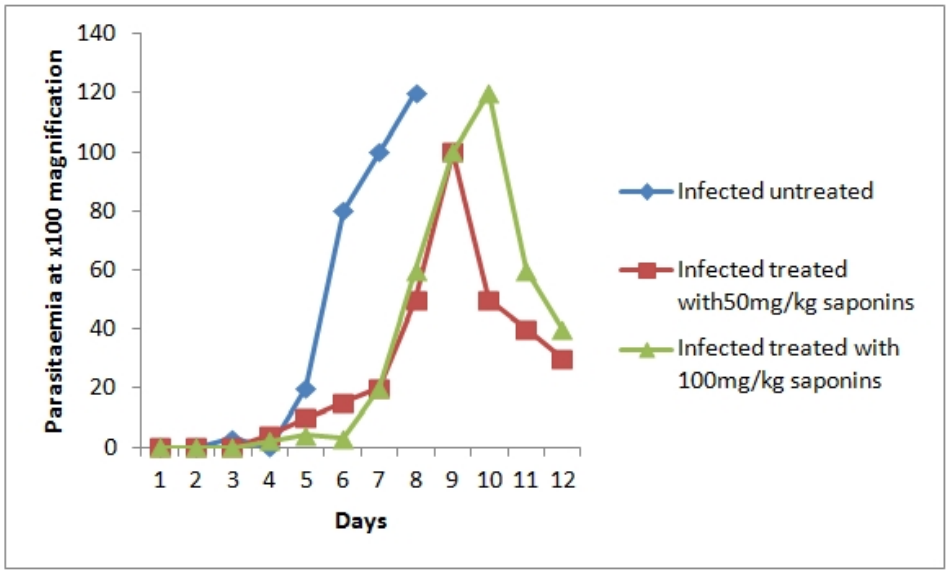

Fig. 4. Parasitaemia levels of *T. b. brucei* infected rats treated with 50mg/kg and 100mg/kg body weight of saponin fraction of *G. kola* seeds. Plotted values are a representation of four determinations

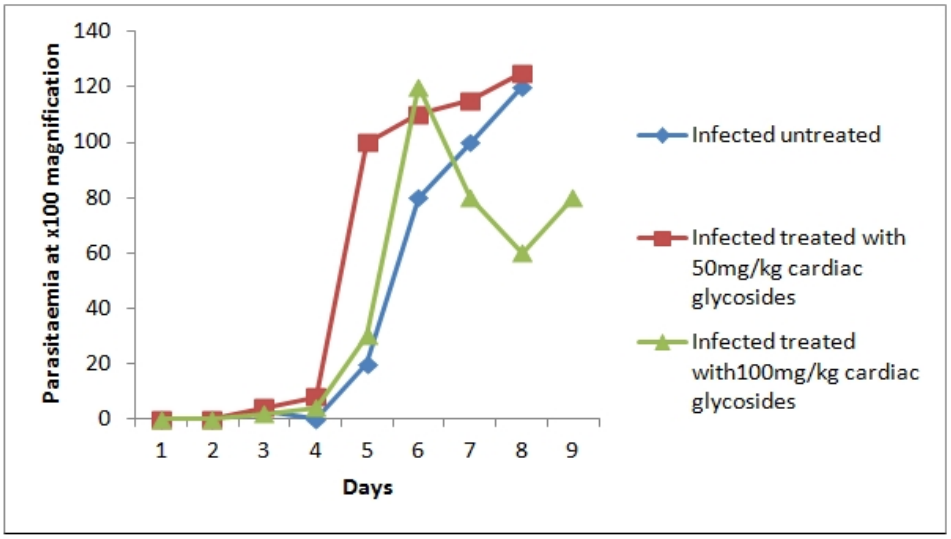

Fig. 5. Parasitaemia levels of *T. b. brucei* infected rats treated with 50mg/kg and 100mg/kg body weight of cardiac glycoside fraction of *G. kola* seeds. Plotted values are a representation of four determinations

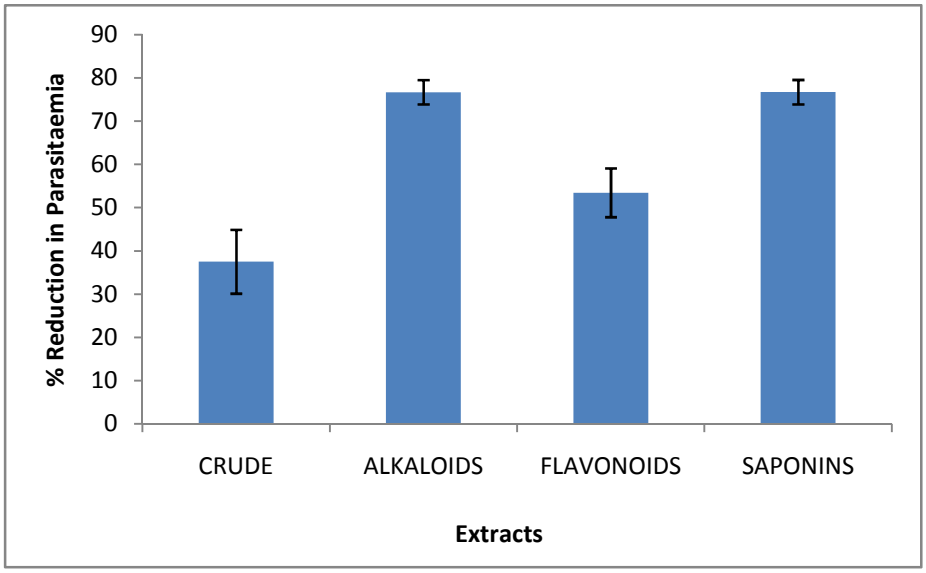

Fig. 6. The percentage (%) reduction in Parasitaemia of *T. b. brucei* infected rats treated with 50mg/kg ethanolic crude extracts and Phytochemical fractions of *G. kola* seeds. n=4±SD.

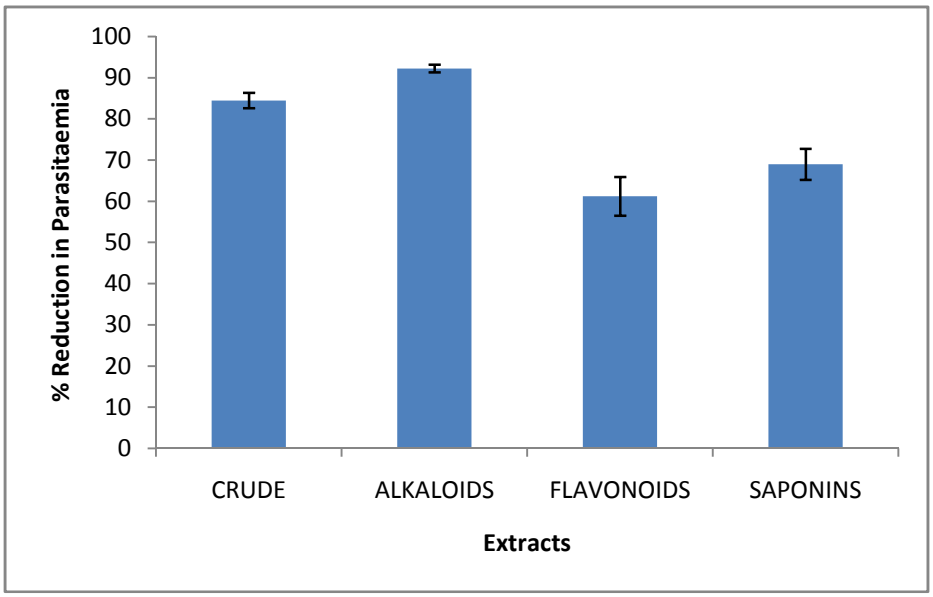

Fig. 7. The percentage (%) reduction in Parasitaemia of *T.b brucei* infected rats treated with 100mg/kg ethanolic crude extracts and Phytochemical fractions of *G. kola* seeds. n=4±SD.

3.3 Effects of Crude Extract and Phytochemical Fractions of *G. kola* Seeds on the Packed Cell Volume (PCV) of Infected Rats

At 50mg/kg, the crude extract and fractions of *G. kola* seeds brought about a significant increase (p=.05) in the PCV of infected rats when compared with the untreated ones especially by day 8 of infection. The mean PCV value of infected rats treated with 50mg/kg flavonoid fraction was within the same range with that of the uninfected untreated group on day 4 and very close to normal on day 8 (Table 1). At 100mg/kg, significant increases (p=.05) in PCV values were observed in all the treated groups by day 8 when compared with the untreated ones. The highest improvement was observed in the group treated with flavonoid fraction. The mean PCV values were within the same range with those of the uninfected untreated groups on both days (Table 2).

Table 1. PCV Levels of *T. b brucei* Infected Rats Treated with 50mg/Kg of Crude Extracts and Phytochemical Fractions of *G. Kola* Seed

Treatment Days	UU	IU	IC	IA	IF	IS	ICg
4	50.25 ±8.73^{ab}	38.50 ±1.91^{cd}	43.75 ±4.79^{bc}	36.00 ±3.27^{d}	52.00 ±2.53^{a}	44.25 ±4.99^{bc}	45.00 ±3.56^{abc}
8	54.25 ±6.50^{a}	21.75 ±3.30^{d}	31.25 ±8.88^{c}	34.75 ±5.25^{c}	45.00 ±2.16^{b}	36.00 ±5.89^{c}	30.00 ±4.55^{c}

n=4±SD; UU= Uninfected untreated (control 1), IU= Infected untreated (control 2), IC, IA, IF, IS and ICg =Infected rats treated with Crude extract, Alkaloid, Flavonoid, Saponin and Cardiac glycoside fractions respectively. Values carrying superscripts different from the control are significantly different at P = .05. PCV values are expressed in percentage (%).

Table 2. PCV levels of *T. b brucei* infected rats treated with 100mg/kg of Crude Extracts and phytochemical fractions of *G. kola* seeds

Treatment Days	UU	IU	IC	IA	IF	IS	ICg
4	50.25 ±8.73^{ab}	38.50 ±1.91^{e}	48.75 ±4.79^{abc}	40.00 ±5.72^{de}	56.25 ±1.50^{a}	42.00 ±2.16^{cde}	47.00 ±3.56^{bcd}
8	54.25 ±6.50^{a}	21.75 ±3.30^{d}	40.75 ±7.63^{b}	30.00 ±5.09^{c}	50.00 ±1.63^{a}	32.00 ±2.16^{c}	30.00 ±4.97^{c}

n=4±SD; UU= Uninfected untreated (control 1), IU= Infected untreated (control 2), IC, IA, IF, IS and ICg = Infected rats treated with Crude extract, Alkaloid, Flavonoid, Saponin and Cardiac glycoside fractions respectively. Values carrying superscripts different from the control are significantly different at P = .05. PCV values are expressed in percentage (%).

4. DISCUSSION

The beneficial effects of medicinal plants depend on the presence of phytochemical compounds which produce definite biochemical and physiological actions in an organism [23]. Approximately one-half of all licensed drugs that were registered worldwide in the 25 year period prior to 2007 were natural products or their synthetic derivatives [24]. Recent efforts on ethnopharmacology has revealed several medicinal plants as potential trypanocides [25,26,27], and natural products such as alkaloids, terpenes, quinones, and polyphenols have been shown to exhibit trypanocidal activities [28].

The ethanolic crude extract of *G. kola* seed exhibited *in vivo* trypanocidal activity in line with the in vitro work earlier reported [19]. The trypanocidal activity of the extract could be linked to the presence of bioactive compounds, some of which were detected in this study. Reports on phytochemical assays of medicinal plants have shown that, antitrypanosomal activity is due to minor components or synergistic interaction of all or some of the active components [19]. The *in vivo* trypanocidal activity of the methanolic extract of *G. kola* seeds had been reported, which revealed the great trypanostatic potential of the plant. It was speculated that the extract exerts the remarkable trypanostatic effect by interfering with cell cycle progression in the parasite, possibly causing cell cycle arrest and thereby halting cell proliferation [29].

The highest trypanocidal activity was observed in the alkaloid fraction in accordance with the *in vitro* work earlier reported (19). The antitrypanosomal property of alkaloids has been suggested to be due to DNA intercalation in combination with protein biosynthesis inhibition [30,31].

The trypanocidal activity exhibited by the saponin fraction may be as a result of cytotoxicity. Saponins are natural glycosides which possess a wide range of pharmacological properties including cytotoxic activity. Their cytotoxic effects have been suggested to be due to either apoptosis inducement or non-apoptotic cell death stimulation. Cell death may be as a result of mechanisms like - stimulation of autophagic cell death, decrease in NO production in cells, or cytoskeleton integrity disassembly [32].

The *in vivo* trypanocidal activity exhibited by the flavonoid fraction in this study is in contrast to the result obtained in the *in vitro* work earlier reported. The flavonoid fraction showed little or no effect on trypanosomes *in vitro* [19]. Although flavonoids have been reported to possess antiviral, anti-allergic, antiplatelet, antiinflammatory, antitumor and antioxidant activities (8), the trypanocidal activity of the flavonoid fraction which was found to be present *in vivo* but absent *in vitro*, suggests that this fraction might be acting through its antioxidant and free radical scavenging properties. This could further be justified by the high improvement in the PCV of infected rats as a result of treatment with the flavonoid fraction. Antioxidants protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Cellular damage and consequently anaemia which is characterized by a significant reduction in PCV is one of the major pathologic effects of trypanosome infection [18]. The antioxidant property of flavonoids might therefore be of great benefit in the fight against this debilitating disease.

The cardiac glycoside fraction though was effective against *T. b. brucei in vitro* [19], had little or no effect on the parasitaemia of infected rats at 50 and 100mg/kg. Although higher dosages may be further attempted, the little or no activity exhibited at these dosages might be as a result of other factors which probably inhibit its action *in vivo*. It is also possible that the phytochemical require the synergistic action of other chemical compounds for its activity to be exerted *in vivo*. In contrast to synthetic pharmaceuticals based upon single chemicals, many phytomedicines have been observed to exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process [23].

5. CONCLUSION

The ethanolic crude extract of *Garcinia kola* seeds and its alkaloid, flavonoid and saponin fractions at 50 and 100mg/kg demonstrated activities against *Trypanosoma brucei brucei* in rats. Further studies will however be required to ascertain the mechanism of antitrypanosomal action of these and other phytochemicals for the possible development of new, safe and effective drugs against human and animal trypanosomiasis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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