ISSN No:-2456-2165

Anti-trypanosomal Activities and Effect *Garcinia kola* Aqueous Seed Extract in Trypanosomiaisis Induced Wister Rat

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Abstract:- This study investigated trypanosomal activity, haematological and lipid profile effects of *Garcinia kola* aqueous seed extract and Berenil drug in typanosomiasis induced Wistar rat. The extract was obtained through Maceration method with distilled water, *In vitro* activity of the extract and Berenil drug was determined using rapid matching techniques at 3 hours post incubation period with different doses of the extract and 3.5mg of Berenil drug. *In vivo* study was done using 62 Wister rats, divided into 10 groups. Group A (normal control): non infected and non treated, Group (B-H) were infected by intra peritoneal inoculation of 1mL of parasitized blood containing 1.0×10^5/ml of *T. brucei gambianse* parasites and treated with different doses of *Garcinia kola* seed extract, 200 – 1400mg, Group I: infected and treated with standard drug dose, Group J: infected and non treated (Pathological control). The treatment lasted for 21 days at 3 days interval. Hematological and lipid profile effects were determined by colorimetric method. Data were analyzed statistically using Anova at 0.01 significant differences. Various hematological and lipid profile effects were observed in all the extract treated and control groups. *Garcinia kola* seed extract exhibited trypanosostatic at dose of <400mg and trypanocidal activity at doses > 400mg in both *in vitro* and *in vivo*, compared with trypanocidal activity of standard Berenil drug dose. *Garcinia kola* seed extract produced moderate side effect on haematological and lipids profile parameters of wistar rat at doses <400mg in the extract treated groups when compared with Berenil drug which produced higher toxicity effect on heamatological and lipid profile parameters at standard dose. This study revealed that *Garcinia kola* aqueous seed extract may be used to design drug for the treatment of trypanosomiasis at lower concentration therefore, the use of Berenil drug in human and animal treatment should be discontinued.

Keywords:- *Trypanosomiasis, Trypanosostatic, Wister rat, Berenil, Garciniakola.*

I. INTRODUCTION

Human African trypanosomiasis or sleeping sickness casesd by *Trypanosoma brucei gambiense, Trypanosoma* brucie *rhodesiensie and* this parasite lives and multiply extracellular in blood and tissue fluid of their human host and transmitted by the bite of infected tsetse flies (*Glossina* spp) (Barett *et al*., 2002).

The occurrence of sleeping sickness is restricted to the distribution of tsetse fly which is exclusively found in sub – Sahara Africa between 14ºN and 20ºN (Barett *et al.*, 2002). More than 250 discreet active sleeping sickness foci in 36 African countries are recognized most of which are in rural areas (WHO, 2005). The course of sleeping sickness is different depending on the sub species, infection with *T. b rhodesiense* lead to an acute form of the disease while infection with *T.b gambiense* gives rise to a chronic infection (WHO, 2005). The symptoms of the first stage of the disease, which is defined by the restriction of trypanosome to the blood and lymph system, included fever, headache, gut pain and itching (Abubarkar, 2012 and WHO, 2005).The clinical sign of the second stage of the disease, characterized by the invasion of *trypanosome* into the central nervous system, neurological and endocrinal system, If left untreated, sleeping sickness patient infected with *T.b rhodesense* will die within months whereas those infected with *T. b gambiense* usually survive for several years (Emmanuel *et al.*, 2011). In 2014, WHO expect committee estimated that 60 million people were at risk of trypanosomiasis disease 300,000 cases per year in Africa and fewer than 30,000 case diagnosed and treated (WHO, 2014).Based on this several drugs have disappeared from market, One of the major problems that severely limit trypanosomiasis chemotherapy is the unwillingness of pharmaceutical companies to invest in development of drug against trypanosome for lack of financial incentive and lack of information about many medicinal plant that have the potential activity against trypanosome and their side effect on animal study (Farombi *et al*., 2002).

Garcinia kola locally known as Orogbo in Yoruba," Namijin" goro in Hausa and Akilu in Igbo languages of Nigeria and commonly called bitter kola in English belong to a class of plants described as masticator (Adeyemi *et al*., 2012). It is found mostly in the forest region and grow as a medium sized tree, up to 12m height. The plant is cultivated and distributed throughout West and Central Africa where it is valued for its edible nut. The hard nut is chewed to realize its bitter content which is traditionally believed to be a stimulant of the nervous system and an enhancer of male potency. A wide range of Medicinal uses *of G. kola* as reported in the literature include its use as antiseptic, antimicrobial, antiviral, and inflammatory, purgative, antidote to the effects of strophatus gratus, remedy for guinea worm infection and for the treatment of gastroenteritis, rheunatisim, asthma, menstrual cramp, bronchitis, throat infection, cure head or chest cold, cough, and liver disorder (Lutje *et al*., 2013). Diminazene aceturate (Berenil) is an aromatic diamidine developed by Hoechest for the treatment of bovine trypanosomiasis, however, its apparent low incidence of adverse reaction and significant therapeutic activity has led some physician in endemic countries to use it extensively for human sleeping sickness without relevant literature on its side effect on animal study since is an agent licensed for venternary use. Therefore, the main aim of this study was to carry out the comparative study of trypanosomal activity and effect of *Garcinia kola* aqueous seed extract and Berenil drug on Biochemical and Liver histology of trypanosomiasis induced albino wister rat , which is a preliminary report towards drug development.

II. MATERIALS AND METHOD

➢ *Collection and Identification of Plant Materials*

Garcinia kola (Bitter kola) seed was purchased from Relief Market in Owerri Municipal in Imo State, Nigeria and identified by a Botanist in the Department of Biological Sciences of Chukwuemeka Odumegwu Ojukwu University, Uli without voucher specimen number.

➢ *Plant Preparation*

Eight (800) hundred pieces *of Garcinia kola* seed was prepared by peeling the coating and chopping the seed into small pieces to allow easy drying at room temperature. The dried pieces were grinded into powder form using a pestle and mortar and stored in a plastic bottle until required for use.

➢ *Drug Source*

The Diaminiezine Acurate (Berenil) was imported from India through Biotan Hong Kong Co, Limited and it was identified and confirmed by a Pharmacist from Federal Polytechnic Nekede Owerri Imo State Nigeria.

➢ *Trypanosome*

Trypanosome brucei gambiense was obtained from stabilates maintained at Nigeria Institute of Trypanosomiasis and Onchocerciasis Research and Control Vom Plateau State, Nigeria thereafter it was maintained in the Biological Science Laboratory of COOU Uli by continuous passage of infected blood into healthy rats.

➢ *Animals*

Albino Wister rats weighing 152 – 250 grams were purchased from Pharmaceutical Technology Department of Federal Polytechnic Nekede Owerri Imo State, Nigeria. The experiment was conducted in compliance with the Canadian Council on Animal Care (CCAC, 1997) Guideline on Animal Used Protocol Review.

➢ *Preparation of Aqueous Extract of the Plant Material*

250 grams of dried powdered *Garcinia kola* seed was dissolved in three liters of distilled water and stirred vigorously at interval of 1 hour for four hours, and allowed to stand on bench for an hour without disturbed. The solution was then refrigerated for twenty four hours and sieved with a laboratory sieve of 0.5µ size, and allowed to stand for one hour to allow its heavy particles to settle down then the supernatant was decanted and filtered using whatman filter paper and the residue was transferred into an open tray and dried in the oven at 100ºC for 2 days scraped with a spatula and grinded into fine powder using laboratory pestle and mortar (Igboli *et al*., 2011). Percentage yield was calculated using the formula.

$$\%\ yield = \frac{weight\ of\ extract}{weight\ of\ powdered\ seed} \times \frac{100}{1}$$

➢ *Acclimatization of Animals*

A total of 66 (sixty six) Albino wistar rats was housed in a clean iron cage well ventilated with standard housing condition. Temperature of 28 – 37ºC, photo period of 12 hours, humidity 50 – 55 %, used for this study, the animals were allowed for free access to poultry feed and tap water, the cage was cleaned on daily bases. The animal was acclimatized for 4 weeks before the commencement of the study. The wistar rats was completely randomized into 10 groups A B C D E F G H I and J comprising of 6 animals each and four (4) animals for acute toxicity study in separate cage (Atawodi, 1998).

➢ *Infection of Animal*

Blood from a highly parasitized rat under anestherzation was obtained from cardiac puncture using syringe and needle. The blood was collected into a blood specimen collection bottle contained ethylene- di-aminetetra acetic acid (EDTA), and diluted with dextrose saline solution using serial dilution method up to 10^5 to serve as inoculums. Healthy rats was inoculated intraperitoriously with 1.0 mL of the inoculums which contain about 1.0×10^5 trapanosome/ml. (Emmanuel *et al*., 2011)

➢ *Study Design*

Ten (10) groups consisting of six (6) healthy albino rats was set up, Group A was not infected, well feed and treated with 10 mL of distilled water to serve as normal control, Group B, C, D, E, F, G and H were infected and treated with different graded doses of *Garcinia kola* seed aqueous extract, 200 mg, 400 mg, 600 mg, 800 mg, 1000 mg, 1200 mg and 1400 mg per kilogram body weight at 3 days intervals of post infection, Group I was infected and treated with 3.5 mg/kg/bw of standard Berenil drug (Diaminazene aeceturate) to serve as

standard control, Group J was infected and untreated to serve pathogenical control.

The treatment of the treated groups and control groups were lasted for 21 days consecutively through oral route of administration for the plant seed extract and intra muscular injection with standard drug thereafter, the trypanosomal activity and the effect of *Garcinia kola* aqueous seed extract and Diamazene aceturate was determined by Biochemical, and Histological parameter examination using standard method

Groups	No. of Rats	Group Name	Group Treatment
A	6	Normal Control	10 ml of distilled water
B	6	Test	200 mg/kg/bw of extract
C	6	Test	400 mg/kg/bw of extract
D	6	Test	600 mg/kg/bw of extract
E	6	Test	800 mg/kg/bw of extract
F	6	Test	1000 mg/kg/bw of extract
G	6	Test	1200 mg/kg/bw of extract
H	6	Test	1400 mg/kg/bw of extract
I	6	Standard control	3.5 mg/kg/bw of Diamaziene acetulate
J	6	Pathological control	Infected non treated

Table 1: Groups, number of rats and types of treatment

❖ *Determination of Oral Acute Toxicity limit test dose of the Extract*

Acute oral toxicity study was determined using limit test dose of 2000 mg/kg/bw according to Organization of Economic Co-operation Development (OECD) Guideline for Testing of Chemical Using Rat or Mice (OECD, 2001). Four albino rats separately received orally 2000 mg/kg/bw of the aqueous extract of *Garcinia kola* seed. The rats were observed continuously for 1 hour after administration of the extract intermittently for 4 hour over a period of 24 hours for 10 days for gross behavioral changes and other sign of toxicity manifestation. The acute toxicity study indicated that there were no visible signs of acute toxicity and death was not observed at the limit dose test of 2000 mg during the 10 day observation period of the animals.

➢ *Determination of Trypanosomal Activity*

The experimental animals was examined for daily death, and the tail vain blood smear was performed at interval of 3 days before treatment, to determine the parasite load as the effectiveness of the treatment based on the day's dosage for both extract and the standard drug.

➢ *Method*

About 20µm of blood will be placed on a grease free slide, then cover with cover slip and examined under microscope at ×10 and ×40 for motile trypanosome and direct smear count will be carried out both for the *G. kola* extract and the Berenil drug treated animal respectively.

➢ *In vitro Study of Aqueous Extract of Garcinia Kola Seed and Berenil Drug on Trypanosoma brucei gambiase Parasite*

Fourty (40) test tubes were used. The test tubes were divided into groups (A – H) of five test tubes each. Cardiac blood collected from one of the donor rats at the peak of parasitemia (250×10^6/ml) was diluted serially with 10 ml of dextrose saline. The aliquots of 0.5 ml containing 5.0×10^5/ml of the parasite were the then pipette into each of the test tubes in Group (A – H). The test tube A was treated with 200 mg/ml, Group B with 400 mg/ml, Group C with 600 mg/ml, Group D with 800 mg/ml, Group E with 1000 mg/ml, Group F with 1200 mg/ml, Group G with 1400 mg/ml of the extract and Group H with 3.5 mg/kg of Diaminizene aceturate per 12.0 ml of distilled water. All the test tubes were incubated at 37°C room temperature. The level of parasitemia in the test tubes were determined at 3 hours intervals for a period of 24 hours using rapid matching technique of Herbert and Lumsden (1976) to count the number of parasite per field under the light microscope at X40, since motility constitutes a relatively reliable indicator of viability among most zooflagenate parasite (Peter *et al*., 1976). Cessation or drop in motility of the parasite was used as a measure to evaluate the anti trypanosomal effect of the plant extract and standard drug (Berenil) under *In vitro* condition.

➢ *In vivo Study of Aqueous Extract of Garcinia Kola Seed and Berenil Drug on Trypanosoma brucei gambiase Parasite*

Animals were observed for daily death and the tail vein blood smear was performed at 3 days interval of post infection treatment to determine the parasite load as the effectiveness of the treatment based on the dosage of both extract and the standard drug.

➢ *Method*

20 µm of blood was placed on a greased free slide, then covered with cover slip and examined under microscope at x10 and x40 objective lens for motile trypanosome and direct smear count was carried out using Herbert and Lumesden standard method of rapid matching counting technique of 1976.

➢ Screening of *Garcinia Kola* Extract and Berenil Drug for Internal Organ Toxicity

- *Blood Samples and Organs Collection*

At the end of the 3weeks of oral administration of *Garcinia Kola* aqueous seed extract and intra muscular injection of the Berenil drug, four rats each from each group

were sacrificed and the blood samples was collected by cardiac puncture, and internal organs were harvested.

The animals were anesthetized with chloroform in desiccators and dissection was carried out to expose the cardiac activity of the heart, blood was collected using sterile syringe and needle and carefully discharged into heparintized bottles and EDTA bottles. The blood sample bottles were labeled according to the groups and the blood samples were analyzed for haematological and lipids profile parameters.

➢ *Determination of haematological parameters*

- Packed Cell Volume: Packed cell volume was determined using micro haematocrit method described by Cheseebrough (2000).
- Hemoglobin concentration: Was determined by Direct readout of haemoglobin using Hb meter
- Mean Cell Hemoglobin Concentration: was carried out by calculation using the formular;

$$MCHC = \frac{Hb}{PCV} \times 100$$

➢ Determination of Lipid Profile

- Total cholesterol, high density lipoprotein, (HDL), low density lipoprotein and Triglyceride were determined by colorimeteric method described by Cheseebrough (2000).

➢ Statistical analysis

The result was analyzed for statistical significance using ANOVAs with Turkey's post hoc test in both extract test and standard drug Berenil and evaluated using SPSS version 2.0 software and Microsoft Excel, data were expressed as mean ± SD, n = 6. The $p < 0.01$ was considered as significant difference (Emmanuel et al., 2011).

III. RESULTS

Group	Group Treatment	Initial number of Parasite Mean± SD	3hours Mean± SD	6hours Mean± SD	9hours Mean± SD	12hours Mean± SD	15hours Mean± SD	18hours Mean± SD	21hours Mean± SD	24hours Mean± SD
A	200 mg of the extract	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
B	400mg of extract	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
C	600mg of extract	5.00±0.00	5.00±0.00	4.00±0.00	4.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00
D	800mg of extract	5.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00	3.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
E	1000mg/of extract	5.00±0.00	3.00±0.00	2.50±0.00	1.00±0.00	0.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
F	1200mg/of extract	5.00±0.00	2.00±0.00	1.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
G	1400mg/ of extract	5.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
H (Standard drug control)	3.5mg/kg/bw of diaminizene aceturate	5.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 2: *In vitro* activities of *Garcinia kola* aqueous seed extract and Diaminizene aceturate observed on *T. brucei gambianse* parasite in 24 hours post incubation at 10^5/mL

Group	Group Treatment	Initial level Mean± SD	Day 3 Mean± SD	Day 6 Mean± SD	Day 9 Mean± SD	Day 12 Mean± SD	Day 15 Mean± SD	Day 18 Mean± SD	Day 21 Mean± SD
A Normal control	10 ml of distilled water	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
B	200mg/kg/bw of extract	5.00±0.00	5.00±0.00	7.00±0.00	6.00±0.00	4.00±0.00	3.00±0.00	2.00±0.00	2.00±0.00
C	400mg/kg/bw of extract	5.00±0.00	5.00±0.00	7.00±0.00	5.00±0.00	4.00±0.00	3.00±0.00	2.00±0.00	1.00±0.00

ISSN No:-2456-2165

D	600mg/kg/bw of extract	5.00±0.00	5.00±0.00	7.00±0.00	6.00±0.00	4.00±0.00	3.00±0.00	2.00±0.00	0.00±0.00
E	800mg/kg/bw of extract	5.00±0.00	5.00±0.00	4.00±0.00	3.00±0.00	3.00±0.00	1.00±0.00	1.00±0.00	0.00±0.00
F	1000mg/kg/bw of extract	5.00±0.00	5.00±0.00	3.00±0.00	2.50±0.00	2.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00
G	1200mg/kg/bw of extract	5.00±0.00	4.00±0.00	3.00±0.00	2.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
H	1400mg/kg/bw of extract	5.00±0.00	4.00±0.00	2.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
I Standard control	3.5mg/kg/bw of Diaminizine accturate	5.00±0.00	2.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
J Pathological control	10ml of Dextrose saline solution	5.00±0.00	6.00±0.00	8.00±0.00	10.00±0.00	20.00±0.00	Death	Death	Death

Table 3: *In vivo* activities of *Garcinia kola* aqueous seed extract and Diaminizene aceturate observed on *T. brucei gambianse* parasite at 10^5/mL in Trypanomiasis induced albino rat for 21 days treatment
Note: letters a,b,c,d… denotes the significant differences of the means at p<0.01

Group	Group Treatment	Initial PCV Mean± SD	Day 3 Mean± SD	Day 6 Mean± SD	Day 9 Mean± SD	Day 12 Mean± SD	Day 15 Mean± SD	Day 18 Mean± SD	Day 21 Mean± SD
A Normal control	10 ml of distilled water	54.25 ± 0.50^{a}	54.25 ± 0.50^{a}	55.25 ± 0.50^{a}	56.25 ± 0.50^{a}	55.50 ± 1.00^{a}	56.25 ± 0.50^{a}	56.75 ± 0.96^{a}	56.25 ± 0.50^{a}
B	200mg/kg/bw of extract	47.75 ± 0.95^{b}	47.00 ± 0.82^{b}	46.25 ± 0.50^{c}	42.50 ± 0.58^{c}	43.25 ± 0.50^{c}	43.75 ± 0.50^{c}	44.75 ± 0.96^{c}	45.25 ± 0.50^{bc}
C	400mg/kg/bw of extract	46.50 ± 0.57^{cd}	43.25 ± 0.96^{ef}	31.25 ± 0.50^{f}	31.75 ± 0.96^{f}	32.00 ± 0.82^{e}	33.25 ± 0.96^{f}	39.25 ± 0.50^{d}	43.75 ± 0.96^{cd}
D	600mg/kg/bw of extract	48.25 ± 0.50^{b}	47.00 ± 0.00^{b}	36.25 ± 0.50^{e}	35.25 ± 1.50^{e}	37.50 ± 1.29^{d}	38.25 ± 0.50^{d}	38.75 ± 0.96^{d}	42.50 ± 1.29^{d}
E	800mg/kg/bw of extract	45.50 ± 0.57^{de}	44.75 ± 0.96^{cd}	42.75 ± 0.50^{c}	43.25 ± 1.50^{c}	43.75 ± 0.50^{c}	45.25 ± 0.50^{b}	47.25 ± 0.50^{b}	46.75 ± 0.96^{b}
F	1000mg/kg/bw of extract	47.25 ± 0.50^{bc}	45.75 ± 0.96^{bc}	44.00 ± 0.82^{c}	45.75 ± 0.96^{b}	46.50 ± 1.73^{b}	47.25 ± 0.50^{a}	47.00 ± 0.82^{b}	47.00 ± 0.82^{b}
G	1200mg/kg/bw of extract	44.25 ± 0.50^{e}	42.25 ± 0.50^{f}	45.25 ± 0.50^{c}	47.00 ± 0.82^{b}	48.25 ± 0.50^{b}	47.50 ± 1.00^{a}	47.50 ± 0.58^{b}	47.75 ± 1.26^{b}
H	1400mg/kg/bw of extract	46.25 ± 0.50^{cd}	44.25 ± 0.50^{de}	46.00 ± 0.00^{c}	47.25 ± 0.96^{b}	48.00 ± 0.82^{b}	48.00 ± 0.82^{a}	48.00 ± 0.82^{b}	47.75 ± 1.26^{b}
I Standard Control	3.5mg/kg/bw of Diaminizine accturate	47.00 ± 0.81^{bc}	46.25 ± 0.50^{b}	41.75 ± 4.86^{de}	39.00 ± 1.41^{d}	37.25 ± 1.26^{d}	36.25 ± 0.50^{e}	36.25 ± 0.50^{e}	37.25 ± 2.50^{e}
J Pathological Control	10ml of Dextrose saline solution	47.50 ± 1.29^{bc}	44.25 ± 0.50^{de}	38.75 ± 0.50^{ef}	28.75 ± 0.96^{g}	28.25 ± 0.50^{f}	0.00 ± 0.00^{g}	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}

Table 4: Effect of *Garcinia kola* aqueous seed extract and Diaminizene aceturate on Haematological index (Packed Cell Volume %) of *T. brucei gambianse* post infection treatment in rat
Note: letters a,b,c,d… denotes the significant differences of the means at p<0.01

Group	Group Treatment	Initial HB Mean± SD	Day 3 Mean± SD	Day 6 Mean± SD	Day 9 Mean± SD	Day 12 Mean± SD	Day 15 Mean± SD	Day 18 Mean± SD	Day 21 Mean± SD
A Normal control	10 ml of distilled water	18.09±0.07^{a}	18.03±0.05^{a}	18.31±0.01^{a}	18.62±0.03^{a}	18.61±0.02^{a}	18.62±0.03^{a}	18.61±0.01^{a}	18.61±0.01^{a}
B	200mg/kg/bw of extract	15.64±0.04^{c}	15.32±0.02^{c}	15.31±0.01^{b}	14.08±0.06^{e}	14.34±0.01^{f}	14.62±0.01^{e}	14.61±0.02^{d}	15.02±0.02^{d}
C	400mg/kg/bw of extract	15.31±0.01^{c}	14.09±0.06^{f}	10.36±0.05^{h}	10.34±0.01^{h}	10.61±0.02^{h}	11.34±0.01^{h}	13.05±0.06^{e}	14.34±0.01^{e}
D	600mg/kg/bw of extract	16.24±0.16^{b}	15.62±0.03^{b}	12.08±0.05^{g}	12.03±0.05^{g}	12.07±0.08^{g}	12.62±0.01^{f}	12.61±0.01^{f}	13.61±0.01^{f}
E	800mg/kg/bw of extract	15.10±0.07^{e}	14.62±0.01^{e}	14.36±0.05^{e}	14.62±0.03^{d}	14.62±0.01^{e}	15.03±0.03^{d}	15.31±0.01^{c}	15.32±0.01^{c}
F	1000mg/kg/bw of extract	15.66±0.05^{c}	15.08±0.09^{d}	14.61±0.01^{d}	15.11±0.12^{c}	15.62±0.01^{d}	15.62±0.01^{c}	16.08±0.10^{b}	16.05±0.06^{b}
G	1200mg/kg/bw of extract	14.50±0.14^{f}	14.07±0.08^{f}	15.09±0.07^{c}	15.34±0.01^{b}	16.15±0.13^{b}	16.01±0.02^{b}	16.06±0.07^{b}	16.01±0.02^{b}
H	1400mg/kg/bw of extract	15.33±0.01^{c}	14.05±0.06^{f}	15.31±0.01^{b}	15.33±0.05^{b}	16.01±0.02^{c}	16.01±0.01^{b}	16.11±0.12^{b}	16.02±0.01^{b}
I (Standard control)	3.5mg/kg/bw of Diaminizine accturate	15.63±0.04^{c}	15.36±0.09^{c}	13.02±0.03^{f}	13.91±0.02^{f}	12.08±0.10^{h}	12.03±0.05^{g}	12.01±0.01^{g}	12.02±0.02^{g}
J (Pathological control)	10ml of Dextrose saline solution	15.30±0.01^{d}	14.63±0.05^{e}	13.03±0.03^{f}	9.36±0.05^{i}	9.33±0.01^{i}	0.00±0.00^{i}	0.00±0.00^{h}	0.00 ±0.01^{h}

Table 5: Effect of *Garcinia kola* aqueous seed extract and Diaminizene aceturate on Heamoglobin concentration (Hb (g/dL)) of *T. brucei gambianse* post infection treatment in rat

Note: letters a,b,c,d… denotes the significant differences of the means at p<0.01

Group	Group Treatment	Initial MCHC Mean± SD	Day 3 Mean± SD	Day 6 Mean± SD	Day 9 Mean± SD	Day 12 Mean± SD	Day 15 Mean± SD	Day 18 Mean± SD	Day 21 Mean± SD
A normal control	10 ml of distilled water [P	33.35±0.01^{a}	33.32±0.01^{a}	33.27±0.01^{b}	33.21±0.01^{b}	33.21±0.01^{e}	33.22±0.01^{d}	33.22±0.01^{de}	33.22±0.01^{d}
B	200mg/kg/bw of extract	33.20±0.01^{c}	33.21±0.01^{b}	33.32±0.01^{a}	32.38±0.01^{b}	33.26±0.01^{cd}	33.20±0.01^{d}	33.19±0.01^{e}	33.32±0.01^{c}
C	400mg/kg/bw of extract	33.33±0.01^{a}	33.32±0.01^{a}	33.32±0.01^{a}	33.32±0.01^{b}	33.32±0.01^{b}	33.13±0.01^{e}	33.23±0.01^{d}	33.23±0.01^{d}
D	600mg/kg/bw of extract	33.27±0.01^{b}	33.31±0.01^{a}	33.32±0.01^{a}	33.32±0.01^{b}	33.32±0.01^{b}	34.05±0.01^{a}	34.06±0.01^{a}	34.05±0.01^{a}
E	800mg/kg/bw of extract	32.97±0.01^{d}	33.16±0.04^{d}	33.19±0.01^{e}	33.21±0.01^{b}	33.32±0.01^{b}	33.31±0.01^{c}	33.31±0.01^{c}	33.32±0.03^{c}
F	1000mg/kg/bw of extract	33.19±0.01^{c}	33.32±0.02^{a}	33.32±0.01^{a}	33.28±0.04^{b}	33.28±0.01^{c}	33.31±0.01^{c}	33.20±0.01^{de}	33.21±0.01^{d}
G	1200mg/kg/bw of extract	33.19±0.01^{c}	33.31±0.01^{a}	33.32±0.01^{a}	33.33±0.01^{b}	33.26±0.01^{cd}	33.32±0.01^{c}	33.33±0.01^{c}	33.32±0.01^{c}

ISSN No:-2456-2165

H	1400mg/kg/ bw of extract	33.26±0.01[b]	33.17±0.01[d]	33.27±0.01[bc]	33.23±0.08[a]	33.96±0.01[a]	33.96±0.04[b]	33.96±0.04[b]	33.98±0.01[b]
I standard control	3.5mg/kg/bw of Diaminizine accturate	33.19±0.01[c]	32.61±0.01[d]	33.25±0.01[d]	33.88±0.46[a]	33.25±0.01[d]	32.88±0.01[f]	32.86±0.03[f]	32.93±0.04[f]
J Pathological control	10ml of Dextrose saline solution	33.26±0.01[b]	33.32±0.01[a]	33.32±0.01[a]	33.53±0.40[ab]	33.22±0.01[e]	0.00±0.00[g]	0.00±0.00[g]	0.00±0.00[f]

Table 6: Effect of *Garcinia kola* aqueous seed extract and Diaminisene aceturate on mean cell hemoglobin concentration (%) of *T. brucei gambianse* post infection treatment in rat

Note: letters a,b,c,d… denotes the significant differences of the means at p<0.01

Group	Group Treatment	TC Mean± SD	TAG Mean± SD	HDL Mean± SD	LDL Mean± SD
A (normal control)	10 ml of distilled water	266.50±2.08[a]	157.25±1.50[a]	146.75±2.75[a]	66.00±1.83[a]
B	200mg/kg/bw of extract	255.50±1.29[c]	155.50±1.91[ab]	143.75±1.71[a]	63.75±1.71[a]
C	400mg/kg/bw of extract	244.00±1.41[d]	153.50±1.29[b]	142.25±1.71[a]	62.25±4.57[ab]
D	600mg/kg/bw of extract	221.50±1.29[e]	145.00±1.83[c]	136.00±0.82[b]	57.75±1.26[c]
E	800mg/kg/bw of extract	215.00±1.41[f]	132.50±1.73[d]	133.25±4.27[bc]	51.75±1.71[d]
F	1000mg/kg/bw of extract	203.50±0.58[g]	127.50±2.38[e]	125.75±1.26[de]	52.25±2.22[d]
G	1200mg/kg/bw of extract	202.75±1.26[gh]	125.25±1.50[e]	122.00±1.41[e]	48.50±0.58[d]
H	1400mg/kg/bw of extract	200.75±0.96[h]	119.00±0.82[f]	116.50±1.91[f]	48.00±0.82[d]
I (Standard control)	3.5mg/kg/bw of Diaminizine accturate	261.50±1.29[b]	154.00±1.41[ab]	143.75±3.77[a]	62.75±2.50[a]
J (Pathological control)	10ml of Dextrose saline solution	121.50±1.29[i]	104.50±1.29[g]	129.25±1.89[cd]	58.25±0.96[bc]

Table 7: Effect of *Garcinia kola* aqueous seed extract and Diaminizene aceturate on Lipid Profile (mg/dL) of *T. brucei gambianse* post infection treatment in rat

Note: letters a,b,c,d… denotes the significant differences of the means at p<0.01

IV. DISCUSSION OF RESULT

In Table 2, *Garcinia kola* seed extract was found to posses the highest *in vitro* activity in 1400 mg/kg/bw by reduced the trypanosome population from 5.0×10^5 to 0.0×10^5 in 6 hours of post incubation period and the least was reduced in 200g that reduced the periodic population from 5.0×10^5 /ml to 4.0×10^5/ml in 24 hours of post incubation period. This observation compared well with that of Berenil the standard trypanocidal drug employed as a control during the study whose mechanism of action has been well established (Barett *et al.*., 2002; Delepaux *et al.*., 2007). The extract of *Garcinia kola* seed has a lower activities (*In vitro*) at 3 hours to 24 hours in lower concentration of 200, 400, and 600 mg. this may be due to the low concentration of active photochemical component of the plant seed in these concentrations but its activities increase in 1000 to 1400 mg from 3 hours to 9 hours post incubation period. This result is in line with the previous study of Bulus *et al.* (2013) who reported that extract of *Moringa olifera* stem and leave had a little effect on the parasite during a two hour of incubation. This variation could be partly contributed to different in type and amount of the photochemical in the various parts of the plant.

When the *Garcinia kola* aqueous seed extract was tested for *in vivo* activities Table 3 the result revealed that reduction in parasitemia load in all the extract concentration test groups, 200, 400, 600, 800, 1000, 1200 and 1400 mg/kg/bw such that 200 mg/kg/bw of the extract reduced the parasitemia from $5.0\times10^5 \pm 0.00$ to $2.0\times10^5 \pm 0.00$ mean ± SD in 21 days which was the lowest concentration that produced trypanostatic effect on the parasite, 1400 mg/kg/bw of the extract shows to be highly effective by reducing the parasite load from $5.0 \times 10^5 \pm 0.00$ to $0.0 \times 10^5 \pm 0.00$ in 12 days post infection treatment in rat which produced a similar trypanocidal effect with 3.5 mg/kg/bw of Diaminizene aceturate at $5.0 \times 10^5 \pm 0.00$ to $0.0 \times 10^5 \pm 0.00$ in 3 days post infection treatment in rats when compared with the pathological control in which the parasitemia increased significantly from $5.0 \times 10^5 \pm 0.00$ to $20.0 \times 10^5 \pm 0.00$ in 12 days post infection treatment until the death of the rats were recorded due to parasitic effect from

day 15 – 21 at ($p<0.01$). This remarkable trypanocidal effect resemble the previous report of the study of Bulus *et al.,* (2015) who reported that *Terminalia avicenniodis* plant extract resulted in significant suppression and total clearance of the parasitemia as observed by 14 days (P.I). This is as good as the complete elimination of the parasite from animal blood seen with Diaminizene aceturate treated group.

The effect of *Garcinia kola* aqueous seed extract and Diaminizene aceturate where also studied on body weight of infected rats and the result (Table 4) showed the highest initial body weight, mean ±SD in normal control group and the lowest in pathological control group at 179.00±3.56 and 153.50±1.91 respectively, there is a significant difference between initial body weight of pathological control and standard control group when compared with the normal group at ($p<0.01$) with the mean ± SD of 153.50±1.91, 167.75±1.50 and 179.00±3.56 respectively. There is a significant difference between the initial body weight, post infection body weight and end of treatment body weight in all the extract treated groups, 200, 400, 600, 800, 1000, 1200 and 1400 mg/kg/bw compared with the normal group and pathological group at ($p<0.01$) which indicated an increase in the body weight of the rat treated with different doses of *Garcinia kola* aquous seed extract and reduction in the weight of the standard treated group and pathological control group. This may be as a result of the toxic effect of the active component of Diaminizene aceturate and the pathogenic effect of *T. brucei gambianse* parasite on the lipid profile of the infected rat. This result is in accordance with the similar study of Abdulazeeze *et al.,* (2013) who reported decease in TC, TAG, HDL and LDL level of infected and none treated rat with *T. brucei brucei* and infected and treated rat with Diaminizene aceturate which resulted into weight loss of the rat and increased in TC, TAG, HDL and LDL level of the rat infected and treated with *Petristroph bicalyculeta* whole plant extract.

The administration of the extract in graded doses to the infected albino rat for 21 days in this study showed the packed cell volume of the normal control group increased generally from day 3 with PCV of 54.25±0.50 to 56.25±0.50 in 21 days and the pathological control group PCV decreased significantly from 47.50±1.29 to 0.00±0.00 in 15 days when the death of the animal occurred in 15, 18 and 21 days respectively, there is a significant difference between the PCV of normal control when compared with the treated groups of 200, 400, 600, 800, 1000, 1200 and 1400 mg/kg/bw and also compared with the standard drug of 37.25±2.50 and significantly difference when compared with the PCV of pathological control group of 0.00±0.00 at ($p<0.01$) (Table 4.5).

The observation of significant decrease in PCV in both the standard drug treated group, and infected untreated control (pathological) group may be indicative of hemolytic anemia associated with the *T. brucei gambianse* infected and Diaminizene aceturate drug. The result is in agreement with the findings of Abubarker *et al.* (2016) who reported that the graded doses of aqueous extract of Fius syconorus linn (Moraceae) Stem and bark increase the PCV of extract treated rat and decrease in PVC of infected untreated rats and standard drug treated group which is an indicative of anemia association with *T. brucei brucei* infection, also this result is in accordance with the previous result of Bulus *et al.,* (2015) who reported that the mean PCV of healthy rat treated strictly with the extract of *Terminalina avicenniodes* (Guiliper) were found to be slightly increase than that of infected and untreated group and standard drug group thus support the presence of hemolytic substance in the substance in the standard drug and not in the extract and the parasite infection with *T. brucei brucei* cause haemolitic anemia .

The result indicated in Table 5 shows that the mean ±SD of hemoglobin concentration (Hb) of the Normal control group increase significantly from 18,30± 0,07 to 18.01 ±0.01 from day 6 to 21days respectively , there is significant increase in the Hb of the extract treated group 200,400,600,800,1000and 1400mg/kg/bw , when compared with the normal control and also compared with standard control with PCV of 12.0±0.00 at $p<0.01$) this suggest that extract of *Garcinia kola* aqueous seed ameliorate the anemia caused by the standard drug and trypanosomiasis infection in untreated group and the standard drug treated group , this result also in agreement with study of Peni *et al.*, 2012; Bulus *et al.,* (2015) who reported that the occurrence of anemia during the infection of *T.brucie* as revealed by drop in Hb concentration of the infected and untreated rats, and infected rat that were treated with (ATA) *Terminalia avicennoides* plant had significantly ($p<0.05$) higher Hb values than those in the positive control (infected and untreated) group indicating the improvement of Hb with ATA extract treatment.

Table 6 revealed that the means standard deviation of Mean cell hemoglobin concentration of normal group is significant to pathological control , and standard control and extract treated group at $p<0.01$, 33.35±0.01, 33.49±0.01 and 33.46± 0.01 respectively. The higher MCHC was recorded in the normal group with 33.35±0.01 while the lowest was observed in pathological untreated group with 33.22±0.01 in 12 days post infection of the rat without treatment. Also there is a significant difference between then mean ±SD of MCHC of 400, and 600mg/kg/bw of extract treated rat in 9 days and 800 and 1000 mg/kg/bw in 9 day post infection treatment of the rat, likewise there is no significance difference between the MCHC of the rat treated with 800 and 1000 mg/kg/bw of the extract in three days of the treatment, Death was also observed in pathological treated control group when the MCHC fell between 33.22±0.01 at $p<0.01$ compared with the normal group in 15, 18 and 21 days this result in away corroborate the study of Shittu *et al.,* (2017) who reported that is a significant decrease $p<0.05$ in the value of Hb, MCHC 3.40 ±0.10 g/dl and 10.20±0.25% of infected not treated group in comparison with the normal control infected early treated and prophylactic treated group.

Table 7 indicated that the highest level of total cholesterol mean ± SD was observed in normal control group 266.50±2.08 and the lowest was in pathological control 121.50±1.29. There is a significance difference between the mean ± SD of the pathological control when compared with

the normal control and standard control group at ($p<0.01$), Also there is a significant difference between all the extract treated group mean±SD of total cholesterol compared with the normal control, standard control and pathological control infected and non treated control group at ($p<0.01$). This similar trend was observed in triglyceride and high density and low density lipo protein of all the infected extract treated group, standard groups and pathological control group as in total cholesterol. This result is in line with the similar result of Abdulazeeze *et al.* (2013) which indicated that the level of total cholesterol (TC), Triglyceride (TAD), high density lipo protein and low density lipo protein cholesterol decreased significantly at ($p<0.05$) in *T. brucei* brucei infected rat compared to normal rat but increased significantly at ($p<0.05$) when the rat were treated with the standard drug and partially purified extract. He also stated that rat given partial purified extract at significantly ($p<0.05$) lowered the level of triglyceride and total cholesterol when compared to untreated normal rat. Also serum level of triglyceride and high density lipo protein in infected rat with partial purified extract were significantly lower than that of normal rat but higher than infected rat at ($p<0.05$). He also stated that there was no significant difference in low density lipoprotein level of normal rat and rats infected in both the standard drug and partially purified extract which proved similar to this present study. Therefore, report has shown that lipids play important role in the pathogenesis of trypanosomiasis (Van *et al.*., 2006; Bala *et al.*, (2011).From the findings of this study it is reasonable to infer that *T.brucie gambianse* infection cause significant decrease in the serum level of cholesterol, HDL, TAG,and LDL. The findings of this study also in line with other report of Abudazeeze *et al.*, (2003). Although I cannot ascertain the reason behind the sudden fall, some pathological mechanisms may be involved .It also reported that lipid serum as an important source of energy for *T,brucie* spp, Van *at al*., (2006). Thus the lowering in serum lipids and cholesterol observed in this study in pathological control (infected and untreated) could partly be the result of *trypanosome* utilization of the molecule, thus the continuous utilization of this molecule from blood stream could be contributory factor to weight loss by the rat and eventually lead to death of some of the animals in this group due to increase in lowering the serum level of lipids and cholesterol and also it is known that blood stream *trypanosome* scavenge blood glucose level for energy Wuroecheke *et al.*(2005) which responsible for hypoglycemia in the trypanosome infected animals.

V. CONCLUSION

Natural products are fast becoming the choice method for the treatment of the trypanosomiasis disease because they are safer and cheaper. This current study revealed that *Garcinia kola* aqueous seed extract contains components that can effectively act on the *Trypanosoma brucie gambianse* parasite as a trypanostatics at lower concentration < 400mg and trypanocidal at concentration > 400mg both in *in vitro* and *in vivo. Garcinia kola* seed extract also prove to produce moderate side effect on haematological and lipids profile index of Albino Wister rat at concentration <400mg and produced severe side effect on both parameters at concentration >400mg, when compared with Diaminizene aceturate which also proved to produce similar level of typanosomal activity, and heamatological and lipids profile effect at standard dose of 3.5 mg/kg/bw.

RECOMMENDATIONS

This findings no doubt is indeed an encouragement for the development of the present and future African chemotherapy that promises succor to a region that has suffered from the debilitating effect of trypanosomiasis and consequently improve the quality of life. There is need for further collaborative study in this area intend to focus on the isolation, spectroscopic characterization and pharmaco - kinetics of the bioactive ingredient in *Garcinia kola* seed which may serve as novel compound in the quest for the development of new affordable and more effective antitrypanocidal therapy.

REFERENCES

[1]. O.S.Adeyemi, M.A. Akanji, and J.T. Ekanem. "Ethanolic extract of *Psidium guajava* influences protain and bilirubin level in *Trypanosome brucei brucei* infected rat". *Journal of Biological Sciences,* **12***:* 111 – 116, 2012.

[2]. A. Abubarkar, E.O. Ogbadoyi, E.O. Ajayeoba, J.I. Okogun, and T.I. Gbodi. "Identification of potential antitrypanosomal compounds in *Tridax procumbens* extract". *International Medical Aromat*ic *Plant*, **10**: 20 – 28, 2012.

[3]. A. Abubarkar and K.Z. Mohammed. "Evaluation of trypanocidal efficacy of aqueous extract of *Ficus sycomorus* Linn. (*Moraceae)* stem bark in albino rats". *Vom Journal of Veterinary Science*, **11**: 58 – 72, 2016.

[4]. M.A. Abdulazeez, A.B. Ibrahim and Z. Edibo."Antitrypanosomal effect of *Perstrophe bicalyculeta* extract on *Trypanosome brucei brucei* infected rat". *Asian Journal of Tropical Biomedicine*, **3:**523-526, 2013.

[5]. S.E. Atawodi. . "Comparative *In vitro* trypanocidal activity of savannah plants in Nigeria". *Afican Journal of Biotechanology,* **3**:177 – 182,1998.

[6]. A.Y. Bala, T. Adamu, V. Abubakar, and M.J. Laden. (2011). "Inhibition of by Extract from *Waltheria indica* Leaf (Sleepy Morning)". *Research Journal of Parasitology*, **6**: 55 – 59, 2011.

[7]. M.P. Barett, D.W. Boykin, R. Brun, and R.R.Tidewell."Human African trypanosomiasis pharmacological, reengagement with a neglected disease". *British Journal of Pharmacology*, **152** (8):115 – 117, 2012.

[8]. T.Bulus.andF.TAddau(2013). "Comparative antitrypanosomal screening of methanolic extracts of *Khaya senegalensis* and *Moringa oleifera". Science World Journal,* **8** (2): 112 – 130, 2013.

[9]. T.Bulus, S. E. Atawodi. And M. Mamman. "Aqueous extract of *Terminalia avicennioides* (guillperr) ameliorates some pathological conditions in *T. brucei brucei* infected rats". *Science World Journal,* **10** (4): 11 – 18, 2015.

[10]. O. Emmanuel, O. Ogbadoyi, Y. Adam, O. Kabiru. and F.O. Roseline. "Preliminary studies of antitrypanosomal activity of *Garcinia kola* nut extract in mice". *Journal of Medicine and Medical Science*s, **2**(1): 628–631, 2011.
[11]. O.O. Emmanuel,Y.K. Adamu, and F.O. Roseline. "Preliminary studies of the antitrypanosomal activity of *Garcinia* kola nut extract in mice infected with *Trypanosome brucei brucei*. *Journal of Medicine and Medical Science*, **2**(1): 628 – 631, 2011.
[12]. E.O. Farombi, O.O. Akani, and G.O. Emerole."Antioxidant and scavenging activity of flavoid extraction (Kolaviron) of *Garcinial kola* seed *In vitro"*. *Journal of Pharmacology Biology*, **40**(2): 107 - 116, 2002.
[13]. J.O. Igoli, A.I. Gray, C.J. Clements, and H.A. Moued. (2011). "Antitrypanosomal activity and cytotoxicity of some compound and extract from Nigeria medicinal plant in phytochemicals". *Bioactivity and Impact on Heath Rasooli*, **5**: 345-358, 2011.
[14]. V. Lutje, J. Seixas, and A. Kennedy. "Chemotherapy for second stage human Africa trypanosomiasis". *The Coherence Data Base of Systemic Review*, **6**:6201 – 6210, 2013.
[15]. C. Monica. (2000). "*District laboratory practice in tropical countries"*. 2nd Edition. Cambridge University Press, Oxford, pp. 376, 2000.
[16]. I.J. Peni, O.P. Ajagbonna, C.M. Elinge, B.M. Agaie, A.T. Elsa, H. Yusuf. and I.M. Ribah. "Effects of *Allium sativum* bulb extract, Diminazene Aceturate and Their combination on parasitaemia, and biochemical indices in rats experimentally infected with *Trypanosoma Brucei Brucei"*. *Journal of Applied Pharmaceutical Sciences*, **02** (06): 112 – 115.2012.
[17]. K.O. Shittu, B. Lawal, A.K. Adeniyi, L.T. Kilani, and R.B. Saka. "Effect of methanol extract of *Musca domestica* larva on some enzymes and haematological prameters in *Trypanosoma brucei brucei* - infected rats". *Nigerian Journal of Basic and Applied Sciences*, **25**(2): 66-74, 2017.
[18]. J.J. Vanhellemond, and A.G.M. Tielens. "Adaptation in lipid metabolism of the protozoan parasite *Trypanosoma brucei brucei"*. *Febs Left*, **580**: 5552 – 5558, 2008.
[19]. WHO (2005). Mortality and health data statistic. Accessed 10 February, 2005.
[20]. A.U. Wurochekke, D.B. James, M.I Bello, and A. Ahmodu. "Trypanocidal Activity of the Leaf of *Guira senegalensis* against *Trypanosoma brucei brucei* Infection in Rats". *Journal of Medical Sciences*, **5**: 1 – 4, 2005.