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Antitrypanosomal Activities, Liver Enzymes and Histological Effects of *Garcinia Kola* Aqueous Seed Extract and Berenil (Diaminizene aceturate) Drug in Trypanosomiaisis Induced Albino Wistar Rat,

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Abstract:- African trypanosomiasis is a major disease of economic and public health importance caused by *Trypanosoma brucei gambianse* parasite that affects humans. In 2014, World health organization stated that 60million people were at the risk of the disease, 300,000 cases were reported in Africa and sub Saharan African countries, 30,000 cases were diagnosed and treated, others died or lives with the disability, based on non availability of novel drugs, very costly, highly toxic, and development of resistance to the drugs by the parasite therefore, the use of plant extract is fast becoming the choice method for the treatment of the disease. This study investigated trypanosomal activity, Liver enzymes and histological effects of *Garcinia kola* aqueous seed extract and Diaminizene aceturate (Berenil) drug in typanosomiasis induced albino Wistar rat. The extract was obtained through Maceration method with distilled water and *In vitro* activity of the extract and Berenil drug was determined using rapid matching techniques at 3 hours post incubation with different doses of the extract and 3.5 mg/kg/bw of Berenil drug. *In vivo* study was done using sixty two (62) albino Wister rats divided into 10 groups. Group A (normal control): non infected and non treated but received 10mL of distilled water; Group (B-H) were infected by intraperitoneal 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, 400 – 1400 mg/kg/bw; Group I: infected and treated with standard drug dose (Standard control); Group J: infected and non treated (Pathological control). The treatment lasted for 21 days at 3 days interval. Liver enzymes effects were determined by colorimetric method. Liver histological analysis was carried out using standard tissue processing and stained with Haematoxyline and Eosin stain. Data were analyzed statistically using ANOVAs Turkeys post hoc SPSS version 2.0 software and at 0.01 significant difference. Results revealed various liver enzyme and histological effects were observed in all the extract treated and control groups. *Garcinia kola* seed extract exhibited trypanosostatic at dose of <400 mg and trypanocidal activity at doses > 400 mg in both *in vitro* and *in vivo*, compared with trypanocidal activity of standard Berenil drug dose at 3.5 mg/kg/bw. *Garcinia kola* seed extract proved to be highly toxic to the liver at high doses >200 mg /kg/bw due to various pathological lesions observed on the liver of the extract treated groups when compared with Berenil drug which also produced similar liver toxicity and enzymes effect at standard dose. *Garcinia kola* aqueous seed extract possesses trypanosostatic and trypanocidal effects in both *In vivo* and *In vitro* and also highly toxic to the liver at high concentration >200 mg/kg/bw. Berenil drug proved to be trypanocidal at standard recommended dose but very deadly due to its toxicity on liver and overdose may lead to lethal effect within 30min of administration. This study revealed that *Garcinia kola* aqueous seed extract may be used to design drug for the treatment of trypanosomiasis at lower concentration and the use of Diaminizene aceturate (Berenil) drug in human treatment should be discontinued due to its high level of liver toxicity and enzyme effect in animal study.

Keywords:- Typanosome, Toxicity, Trypanosostatics, Trypanocidal, Garciniakolaseed, Berenil drug.

I. INTRODUCTION

Human African trypanosomiasis or sleeping sickness disease caused by subspecies of trypanosome, *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

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(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 sub-Sahara African countries ,fewer than 30,000 case diagnosed and treated others died or live with disability(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, the general believed about it apparently 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 ventenary 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 Liver enzymes and 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 100^{o}C 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 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 was 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 was 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 37ºC 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 a desiccator 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 non heparintized bottles and EDTA bottles and the extracted organs were preserved in 10 % formaldehyde solution. The blood sample bottles and the organ bottles were labeled according to the groups and the blood samples were analyzed for biochemical and histological parameters

➢ *Determination of Biochemical function of Liver*

Alanine Aminotransferase was determined using colorimetric method described by (Valentine, 1984)

Alkaline Phosphate was determined using colorimetric method described by (Cheesbrough, 2000).

Aspatate transamylyse was determined using colorimetric method described by (valentine, 1984).

Total Serum billirubin was carried out using the Van den Bengh Diazo reaction method describe by (Valentine, 1984).

Histological examination was carried out by fixing each liver in 10% formalin and undergone a standard tissue processing for histological examination and thereafter a central section of 4µm thickness, were cut from the tissue and stained with heamatoxylin and eosin stain then view at ×100 objective of light microscope.

➢ *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. RESULT

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
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

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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 2: *In vitro* activities of *Garcinia kola* aqueous seed extract and Diaminizene aceturate observed on *T. brucei gambianse* parasite in 24hours 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
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 $p<0.01$

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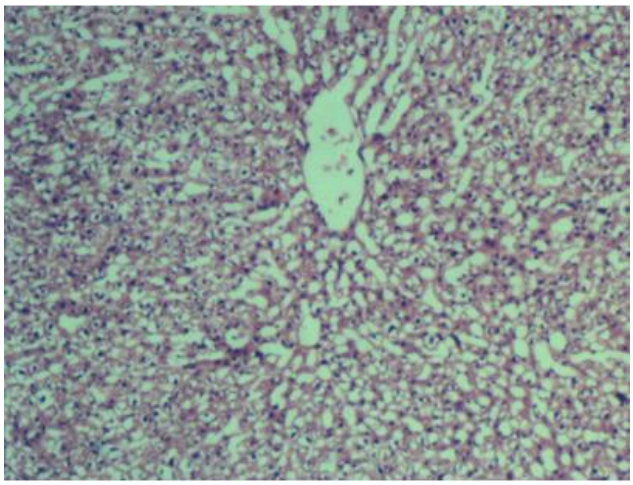
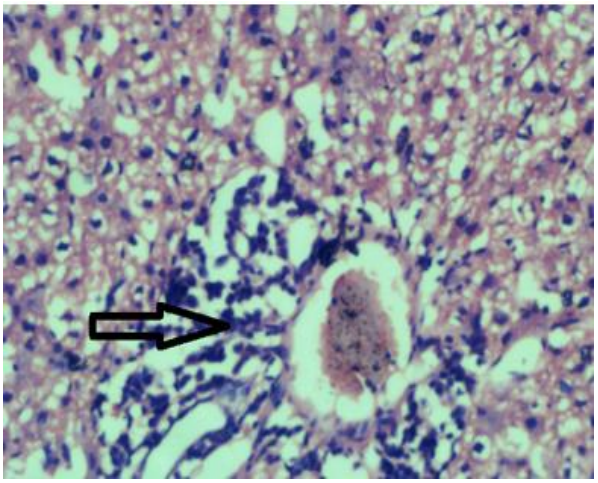
Group	Group Treatment	ALP Mean± SD	AST Mean± SD	ALT Mean± SD	Total Billirubin Mean± SD
A (Normal control)	10 ml of distilled water	92.50 ± 1.29^{a}	12.75 ± 0.96^{def}	17.25 ± 0.96^{b}	0.78 ± 0.01^{d}
B	200mg/kg/bw of extract	72.75 ± 0.96^{e}	13.75 ± 0.50^{cde}	12.75 ± 0.96^{de}	0.65 ± 0.01^{f}
C	400mg/kg/bw of extract	87.75 ± 1.26^{c}	16.25 ± 0.50^{b}	18.50 ± 1.00^{b}	0.88 ± 0.01^{c}
D	600mg/kg/bw of extract	81.50 ± 1.29^{d}	14.75 ± 0.50^{bcd}	18.75 ± 0.96^{b}	0.54 ± 0.02^{h}
E	800mg/kg/bw of extract	73.00 ± 0.82^{e}	11.75 ± 0.96^{ef}	11.50 ± 1.29^{ef}	0.73 ± 0.03^{e}
F	1000mg/kg/bw of extract	75.25 ± 0.96^{e}	12.75 ± 0.96^{def}	13.75 ± 0.96^{cd}	0.76 ± 0.02^{de}
G	1200mg/kg/bw of extract	66.25 ± 1.71^{f}	11.00 ± 1.41^{f}	8.00 ± 0.82^{g}	0.55 ± 0.02^{gh}
H	1400mg/kg/bw of extract	55.75 ± 0.50^{g}	15.25 ± 0.96^{bc}	10.75 ± 0.96^{f}	0.58 ± 0.03^{b}
I (Standard control)	3.5mg/kg/bw of Diaminizine accturate	92.25 ± 0.96^{b}	12.00 ± 0.82^{ef}	14.75 ± 0.96^{c}	1.22 ± 0.02^{a}
J (Pathological control)	10ml of Dextrose saline solution	113.75 ± 3.59^{a}	19.75 ± 1.71^{a}	29.00 ± 0.82^{a}	1.59 ± 0.01^{h}

Table 4: Effect of *Garcinia kola* aqueous seed extract and Diaminizene aceturate on Liver function (IU/L) of *T. brucei gambianse* post infection treatment in rats.



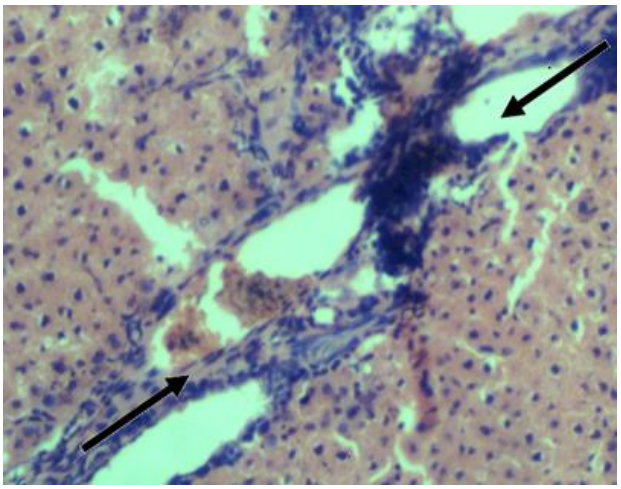
AL

Plate1: Group A Non Infected and Non treated group (normal control). **Liver** showing normal liver architecture with portal vein (H&E stain) x 100



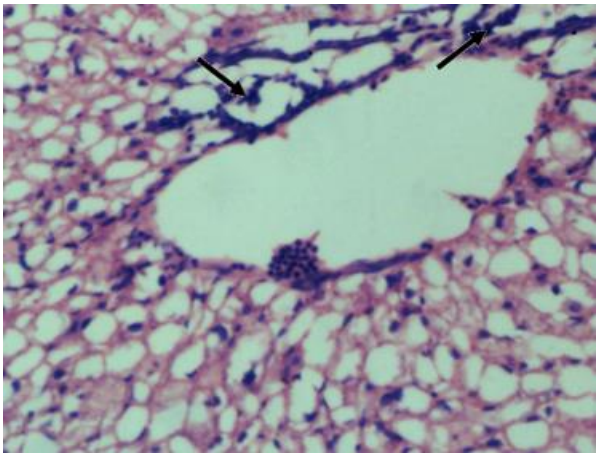
CL

Plate3: The effect of 400 mg/kg/bw of *Garcinia kola* aqueous seed extract on the **liver** of Group C Infected and treated indicated by inflammatory cells around the portal track with arrow (H&E stain) x 100



BL

Plate 2: The effect of the **liver** of Group B (Infected and treated with 200 mg/kg/bw of *Garcinia kola* aqueous seed extract) which indicated inflammatory cells around the portal tract by the arrow (H&E stain) x 100



DL

Plate 5: Effect of 600mg/kg/bw of *Garcinia kola* aqueous seed extract on the **liver** of Group D (Infected and treated) indicated by collection of inflammatory cells around the small vain with

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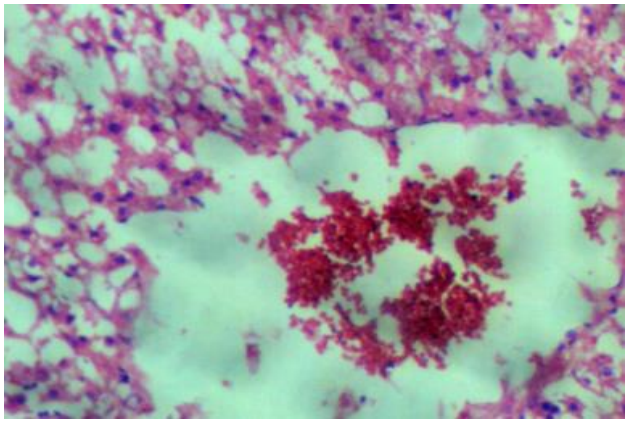
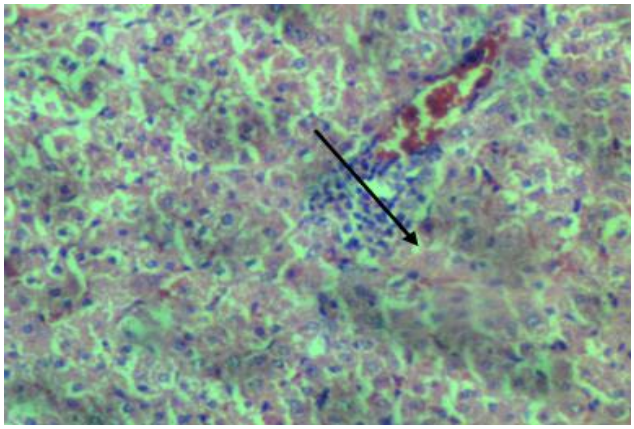


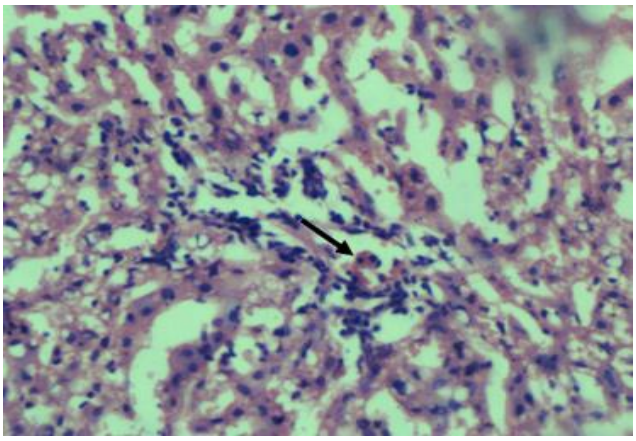
EL

Plate4. The effect of 800 mg/kg/bw of *Garcinia kola* aqueous seed extract on the **liver** of group E (Infected and treated) indicated by blood clots in a central vein with arrow (H&E stain) x 100



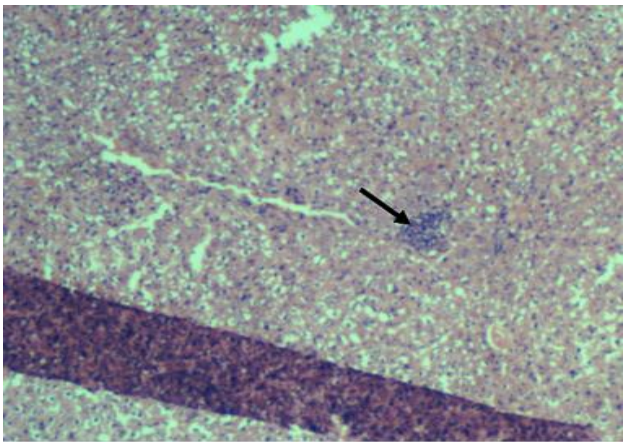
HL

Plate 9: The effect of 1400mg/kg/ bw. Of *Garcinia kola* aqueous seed extract on the **liver** of Group H (Infected and treated) indicated by the Portal inflammation cell with arrow (H&E stain) x 100



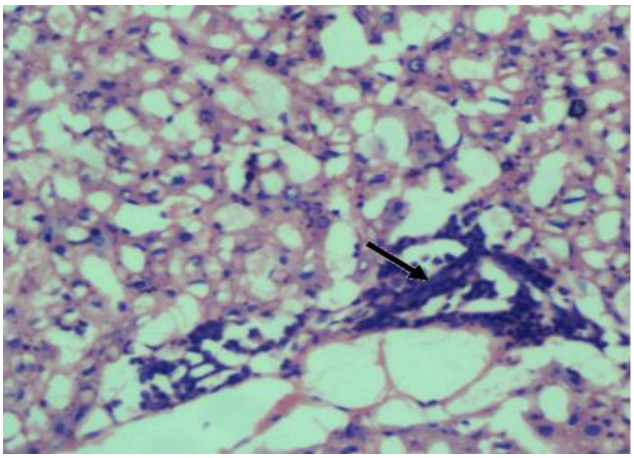
FL

Plate 6: Effect of 1000mg/kg/bw of *Garcinia kola* aqueous seed extract on the liver of Group F (Infected and treated) indicated by the focal inflammation cell with arrow (H&E stain) x 100



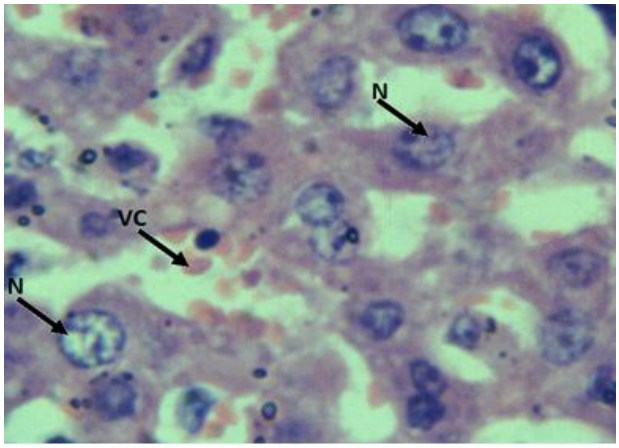
IL

Plate 8: Effect of the Diaminizene aceturate standard drug on **lung** of Group I (infected and treated) indicated by the evidence of focal inflammation masking the hepatocyte with arrow (H&E stain) x 100



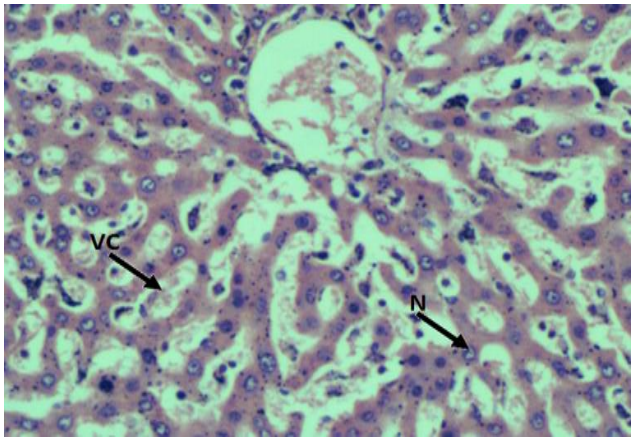
GL

Plate 7: The effect of 1200mg/kg/ bw.of *Garcinia kola* aqueous seed extract on the **liver** of Group G (Infected and treated) indicated by the Portal inflammation cell with arrow (H&E stain) x 100



JL

Plate 10: The effect of *Trypanosoma brucei gambianse* on the **liver** of Group J (Infected and non treated) Pathological control by the evidence of total deranged of general architecture with deformed hepatocyte, necrosis and vascular congestion (H&E stain) x 100



2L

Plate 11: The effect of Diaminizene aceturate drug overdose on the **lung** of (Infected and treated) rat with the evidence of deformed hepatocyte, Necrosis, and Vascular congestion with arrow (H & E stain) x 100

IV. DISCUSSION OF RESULTS

In Table 2, *Garcinia kola aqueous* seed extract was found to posses the highest *in vitro* activity in 1400 mg by reducing the trypanosome population from 5.0×10^5 to 0.0×10^5 in 6 hours of post incubation period and the least was produced in 200mg 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 extract 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 phytochemical 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 inline 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.

Table 4 shows highest level of mean ± SD of Alkaline phosphate was observed in pathological control group at 113.75±3.59 and normal control group 92.05±1.29 and there is no significant difference between the mean ± SD of alkaline phosphate of the normal control and pathological control at ($p<0.01$). Also no significance between the mean ± SD of 800 mg/kg/bw and 1000 mg/kg/bw of extract treated group, 73.00±0.82 and 75.25±0.96 when compared with the normal group at ($p<0.01$). Also, significance was recorded between the extract treated group of alkaline phosphate when compared with the standard and pathological control group. Meanwhile Aspatate transamylase mean ± SD in pathological control is very high compared with extract treated and Bereni drug treated group which showed a significant difference between two when compared with the normal at ($p<0.01$).

The high level of Alkaline transamylase was observed in infected non treated group with the mean ± SD of 8.0±0.00 which shows significant difference between the level of Alanine transamylase in pathological control, standard control when compared with the normal control at ($p<0.01$). The billirubin level was recorded to be very high in pathological control group at mean ±SD of 1.59±0.01 and the lowest was 0.54±0.02 in 600 mg/kg/bw of the extract treated group which shows significance between the mean ±SD of normal control group, pathological control group, standard control group as well as all the extract treated group when compared at ($p<0.01$). this liver function test result is in agreement with the similar result of Shittu *et al.* (2017) which indicated that there was no significant difference ($p<0.05$) in the liver alkaline phosphate activities of the infected treated with standard drug when compared with the control group whereas other groups showed a significant increase. Also serum alkaline phosphate activities of infected non treated group were significantly higher than the other when compared to the control group. Therefore, analysis of serum enzyme have proved to be very important biochemical marker of many diseased state and are related to alter of enzyme activities and measurement of their activities may be diagnostic for many diseases. This disease condition can result in elevation of enzyme activities (Nelson and Cox, 2005). Alkaline phosphate is a protein found in the body tissue such as liver, bile duct and bones, serum (ALP) activities is a very useful serum bio marker indicator of liver disease (Lawal *et al.*, 2016). The significant increase in serum ALP activities in an untreated rat when compared with those treated with the extract and standard drugs comfirmed the early report that infection with *T. brucei gambianse* could gradually affect the liver enzyme and effectively damage the liver by increasing activation of the enzyme molecules in site and release of membrane component including alkaline

phosphate into the extra cellular fluid (Yakubu *et al.*, 2005). However rat treated with the aqueous extract of *Garcinia kola* seed showed ameliorated effect as it caused significant difference in the serum activities of the elevated liver enzyme when compared with pathological control.

The liver showed moderate to marked inflammatory cell around the portal small vain, Focal inflamention masking the hepatocyte, total derange general architecture with deformed hepatocyte, necrosis and Vascular congestion in all the extract treated group, standard drug group treated rat ,Infected and non treated group and accidental drug over dose (7.0 mg/kg/bw. of Diaminizene aceturate autopsy, indicated by the plates1, 2, 3, 4, 5, 6, 7, 8, 9,10 and 11 respectively this result also corroborate the findings of Abubakar *et al*. (2006) who reported marked hepatocellular necrosis following experimental infection with *T, brucei brucei* and treated with 400 mg/kg/bw of aqueous steam bark of extract of ficus sycomorus linn.

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* aqueous seed extract also prove to produce moderate side effect on biochemical index of the liver of Albino Wister rat at concentration <400mg and produced severe side effect on the liver histology at higher concentration >400mg, when compared with Diaminizene aceturate which also proved to produce similar level of typanosomal activity, Biochemical and histological effect on the liver 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 pharmacokinetics 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.

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