

EFFECTS OF HEART CYTOTOXICITY, LIPID PROFILE AND ANTI-TRYPANOSOME *GARCINIA KOLA* PLANT SEED EXTRACT COMPARED WITH STANDARD DIMINIZENE ACETURATE DRUG IN TYRPANOSOMIASIS DISEASE INDUCED ALBINO WISTER RAT.

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Abstract: Trypanosomiasis is a disease of public health importance and one of the most neglected tropical diseases in tropical Africa. It caused by *Trypanosomabruceibruceigambianse* parasite that infects humans in rural and urban settings. The use of plant extract for the treatment of this disease is now widely becoming acceptable. Therefore, this study aimed at investigating the Lipids profile, heart cytotoxicity effect and anti - trypanosomal activities of *Garcinia kola* seed extract compared with Diaminizene aceturate standard drug in trypanosomiasis disease induced albino Wister rat. The maceration method with distilled water was used to obtain the plant extract, and various standard methods were used to carry out photochemical analysis of the plant seed extract. The rapid matching techniques at 3 hours post treatment with the different dosage of the plant extract and the 3.5 mg of standard dose of Berenil drug was used to determine, the *In-vitro* activity of the plant extract and standard drug.. Likewise the study of *in-vivo* activity of the plant extract was determined using sixty two albino rats and sub- divided into 10 groups as A –H and group J was a pathological control, all the treatment lasted for 21 days at interval of 3 days, histological and biochemical effects were determined by colorimetric methods and standard tissue processing and staining with Haematoxylin and Eosin standard method. Data obtained were analyzed using Anova Turkey's post -hoc SPSS statistical package at a significant difference level of 0.01%. Result indicated various photochemical agents of the plant seed such as Flavonoids, Steroids and Tannin and various biochemical lipid profile and heart histological effects was observed in all extract doses of the treated groups and control, *Garcinia kola* seed extract proved to be trypanostatic at a lower concentration of less than 400mg and trypanocidal activity at a concentration higher than 400mg in both *in-vitro* and *in-vivo*, in computation with the activity of the standard drug at concentration of 3.5mg which is trypanocidal this plant seed extract shows to be more toxic to the heart at high concentration greater than 200mg as a result of lesions indicated on the heart tissue of the extract treated groups when compared with standard drug which also produced similar heart toxicity effect at standard dose. Therefore it can be deduced that this plant seed aqueous extract may be utilized into drug development for the treatment of trypanosomiasis disease at low concentration and the administration of Diminazene aceturate (Berenil) drug in treatment human and animal should be discouraged as a result of its high level toxicity to animal heart in this study.

Keywords: Trypanosomiasis, *Garcinia kola*, Cytotoxicity, Lipid profile, Diminazene aceturate

Study background.

In 2005, WHO stated that the sleeping sickness is restricted to where there is high distribution of *Glossinia papalis* known as tsetse fly which is found exclusively in sub – Sahara Africa between 20°N and 14°N (Ekesiobi. *e tal.*, 2024). Approximately More than 350 activediscreet of sleeping sickness foci in 46countries of Africa are mostly recognized in rural areas (Abiodun, *et al.*, 2024).

Trypanosomabrucie- rhodesiense and *T.bgambiense* were found in South, West and East Africa where as *T.bgambiense* occur in mostly in Central Africa both as a different of sleeping sickness and it depends on the sub- species, infection with *Trypanosoma.brucierhodesiense* can eventually lead to an acute form of the trypanosomiasis in animal and human while infection with *Typanosoma.bruciegambiense* gives rise to a severe infection (Abiodun, *et al.*, 2024).The early stage of this disease, which was indicated by the presence of trypanosome parasite in the blood stream, and the signs ranges from headache, fever, itching, spinal and gut pain (Abubarkar, 2012 and WHO, 2005).The pathological sign of the disease second stage is characterized by the presence of *Trypanosome* the central nervous system, endocrine and neurological system of the body, if its left untreated, the patient will die within months whereas those infected with *T. b gambiense* can survive for many years (Abiodun, *et al.*, 2024).In late 19th century, African experienced severe scourge of sleeping sickness disease epidemic such that the most devastating of epidemic is when 300,000 to 500,000 death occurred between 1896 and 1906 which affected the Congo Basin and the Busoga focus in Uganda and Kenya mainly (Stake, 2015; WHO, 2005 ((Abiodun, *et al.*, 2024).

Garcinia kola is also known as bitter kola in English is a class of masticator plant (Adeyemi *et al.*, 2012). It is mostly found in the rain forest region and it grow up to the height of 12 m. The cultivation and distributed of this plant is throughout Central Africa and West Africa where its edible nut is valued for it medicinal use and improvement of male reproductive potency. (Lutje *et al.* 2013 (Emmanuel, *et al.*, 2011).

Diminazene aceturate (Berenil) is adiamidinearomaticdrug that was originally developed in India by Hoechst pharmaceutical company for the treatment of animal trypanosomiasis, disease but with no relevant literature on its effect for it use in human treatment of trypanosomiaais, since is an agent licensed for animal use only and with a general believe about it low incidence of adverse side effect and its therapeutic activity has led to it use by some physician in an endemic countries to use it for treatment of human with sleeping sickness,. Therefore, this study aimed at investigating the Lipids profile, heart histological and anti - trypanosome effect of *Garcinia kola* seed extract compared with standard drug in trypanosomiasis disease induced albino Wister

Materials and Methods.

Plant Seed Source and identification

Bitter kola seeds were obtained from a local market in Owerri in Imo State of Nigeria, and it was identified for the authentication by an Ass. Prof. Ukpaka C.J of the Department of Biological Sciences of Chukwuemeka Odumegwu Ojukwu University, Uli with specimen number REF/BIO/045.

Preparation of the plant seed extract

About Eight (850) hundred and fifty pieces of the plant seed was processed by removing the coating and cut into smaller pieces and air dried at normal room temperature. The dried pieces were alter grinded into powdered form using grinding marching and stored in a clean plastic bottle.

Source of Standard drug

The Diaminiezine aceturae (Berenil) was developed and produced by Heien Chang ShengTang Animal drug Co .LTD and imported from India through Biotan pharmaceutical Co, LTD and identification and confirmation

carried out by Pharmacist in the Biochemistry department of Federal Polytechnic Nekede Owerri Imo State in Nigeria.

Trypanosome

The parasite *Trypanosome bruceigambiense* was collected from stabilized parasite store at Nigeria Institute of Onchocerciasis and Trypanosomiasis Research & Control Vom Plateau State, of Nigeria and it was stored in the COOU Biological Science Laboratory Uli through a continuous transfer of infected blood with the parasite into another healthy rats.

Animals

Albino Wister rats with weight ranges from about 200 – 250 grams was purchased at the Pharmaceutical Technology department of Federal Polytechnic Nekede Owerri in Imo State, of Nigeria.

Aqueous Extract Preparation of the Plant Material

About 350grams of powdered Bitter kola seed was totally dissolved in four liters of distilled water and mixed at interval of 2 hour for 6 hours, and it was allowed to maintained stability on bench for 2 hour without disturbance it was refrigerated for 48 hours and then sieved with a what -man laboratory filter paper of about 0.5in size and allowed to stand for one hour and residue was spread in an open tray and oven dried at 100^oc for 4days, scraped with a spatula and grinded into powdered.(Igboli, *et al.*,2011).

The above formula was used to calculation the %yield

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

Infection of Animal

Highly parasitized rat blood was obtained through cardiovascular vein puncture using syringe and needle and it was collected into a specimen bottle contained ethylene-di-amine tetra acetic acid (EDTA), and later diluted serially with 10% solution of dextrose saline to the turn off 10⁵ to serve as inoculums, using intra peritoneal inoculation of healthy rat (Emmanuel *et al.*, 2011).

Experimental Design

Table .1: Number of rats, Groups and treatment

Groups	No. of Rats	Groups	Treatment
A	6	Control	10 ml solution of distilled water
B	6	Test	200mg of extract
C	6	Test	400 mg of extract
D	6	Test	600 mg of extract
E	6	Test	800 mg of extract
F	6	Test	1000 mg of extract
G	6	Test	1200 mg of extract
H	6	Test	1400 mg of extract
I	6	Standard control	3.5mg of Diamizienacetate
J	6	Pathological control	Infected and untreated

Phytochemical Screening of the aqueous seed extract of the plant

Evans (1996) method was used for most Physiochemical screening while Omwirhimen *et al.* (2010) method was used to screen for only Terpenes and Quinines

Determination of Trypanosomal Activity

About 2.0µm of blood sample collected was placed on dried and a grease free slide, spread and then cover a glass cover slip then examined under microscope at ×10 and ×40 objective for active motile trypanosome and direct smear technique was used to count all the parasite both for the *G. kola* extract and the Diminazene aceturate treated animal.

***In-vitro* Study of the plant aqueous Seed extract and Berenil standard Drug on *Trypanosoma brucei* gambiase Parasite**

Forty Test tubes were used to carry out the procedure, the test tubes were sub- divided into groups labeled A – H with five test tubes each. Cardiovascular venous blood was collected from one of the highly parasitized rats at the peak of the disease ($250 \times 10^6/\text{mL}$) and it was later diluted serially with 10ml of 10% dextrose saline and each small portion of 0.50 mL containing $5.00 \times 10^5/\text{mL}$ of the parasite organism was transferred into each test tube with Groups labeled (A – H). The test tubes A-H were treated with 200 - 1400 mg/mL of the extract in different groups and with 3.5 mg/kg of Berenil drug per 10.0 mL of distilled water the test tubes were incubated at standard room temperature of 37°C . The level of parasitemia in all the test tubes were determined at the 3 hours intervals for a period of 24 hours using standard method Lumsden and Herbert of 1976 to determine the total number of parasite per field under the light microscope at ×40, (Abiodun, *et al.*, 2018). Reduction or stoppage in mobility of the parasite was used to evaluate the anti-trypanosomal activity of the plant seed extract and standard drug under *In-vitro* condition.

***In-vivo* Study of the plant Aqueous Seed Extract and Berenil standard Drug on *Trypanosoma brucei* gambiase Parasite**

About 2.0 µm of blood was placed on a dried and greased free slide, then later covered with a glass microscope cover slip and viewed under microscope at ×40 and ×10 high power objective lens for motile trypanosome and direct smear was used to count all the parasite.

Screening of the plant seed Extract and Berenil Drug for Heart Toxicity

Heart and Blood Samples Collection

At the end of the 3 weeks of the plant aqueous seed extract oral administration and intra- muscular injection of the standard drug, four rats each were sacrificed from each group and the blood samples were collected by cardiovascular puncture, and heart organs were obtained when the animals were under the anesthetization condition using chloroform in a desiccator and the animal dissection was done to expose the cardiac heart function, the samples of the blood were obtained using sterile needle and syringe into non-heparinized bottles and E.D.T.A bottles and the organs extracted were preserved in 10.0 % formalin solution. All the blood sample bottles and the organ sample were labeled accordingly and the blood samples were analyzed for biochemical parameters for lipid profile, Total cholesterol (TC), Triglyceride (TAG), High density lipoprotein (HDL), Low density lipoprotein (LDL) using colorimetric method and histological examination was carried out with

standard tissue processing method and stained with heamtoyxlin and eosin stain technique wish was developed by Mayer in 1972.

Statistical Analysis

All the data obtained were expressed as a $\pm$ SD of mean from four rats from the group at the end of the treatment. The analysis of the data was carried out with the use of ANOVAs Turkey's post hoc test for the extract test and the standard drug. The significant difference was compared by using normal control and pathological control in Trypanosomal activity. All statistical data was analysis and evaluated with SPSS version 2.0. And any p -value of <0.01 was considered as statistically significant difference (Emmanuel *et al.*, 2011).

RESULTS

Table 2: the *in-vitro* activities of Diaminizenacetate drug and the plant seed aqueous extract observed on *Trypanosoma. bruceigambianse* parasite after 24 hours incubation at $10^5/\text{mL}$

Group	Treatment of the groups	Number of Parasite initial $\pm$ SD of Mean	3hours $\pm$ SD of Mean	6hours $\pm$ SD of Mean	9hours $\pm$ SD of Mean	12hours $\pm$ SD of Mean	15hour $\pm$ SD of Mean	18hours $\pm$ SD of Mean	21hours $\pm$ SD of Mean	24hours $\pm$ SD of Mean
A	200 mg of the extract	5.0 $\pm$ 0.00	5.0 $\pm$ 0.00	5.0 $\pm$ 0.00	5.0 $\pm$ 0.00	4.0 $\pm$ 0.00	4.0 $\pm$ 0.00	4.0 $\pm$ 0.00	4.0 $\pm$ 0.00	4.0 $\pm$ 0.00
B	400mg of the extract	5.0 $\pm$ 0.00	5.0 $\pm$ 0.00	5.0 $\pm$ 0.00	5.0 $\pm$ 0.00	4.0 $\pm$ 0.00	4.0 $\pm$ 0.00	4.0 $\pm$ 0.00	4.0 $\pm$ 0.00	4.0 $\pm$ 0.00
C	600mg of the extract	5.0 $\pm$ 0.00	5.0 $\pm$ 0.00	4.0 $\pm$ 0.00	4.0 $\pm$ 0.00	3.0 $\pm$ 0.00	3.0 $\pm$ 0.00	3.0 $\pm$ 0.00	3.0 $\pm$ 0.00	3.0 $\pm$ 0.00
D	800mg of the extract	5.0 $\pm$ 0.00	4.0 $\pm$ 0.00	4.0 $\pm$ 0.00	4.0 $\pm$ 0.00	3.0 $\pm$ 0.00	2.0 $\pm$ 0.00	2.0 $\pm$ 0.00	2.0 $\pm$ 0.00	2.0 $\pm$ 0.00
E	1000mg/of the extract	5.0 $\pm$ 0.00	3.0 $\pm$ 0.00	2.50 $\pm$ 0.00	1.0 $\pm$ 0.00	0.0 $\pm$ 0.00	1.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00
F	1200mg/of the extract	5.0 $\pm$ 0.00	2.0 $\pm$ 0.00	1.0 $\pm$ 0.00	1.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00
G	1400mg/ of the extract	5.0 $\pm$ 0.00	1.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00
H (Standard drug control)	3.5mg/kg/b w of diaminizen eacetate standard drug	5.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00

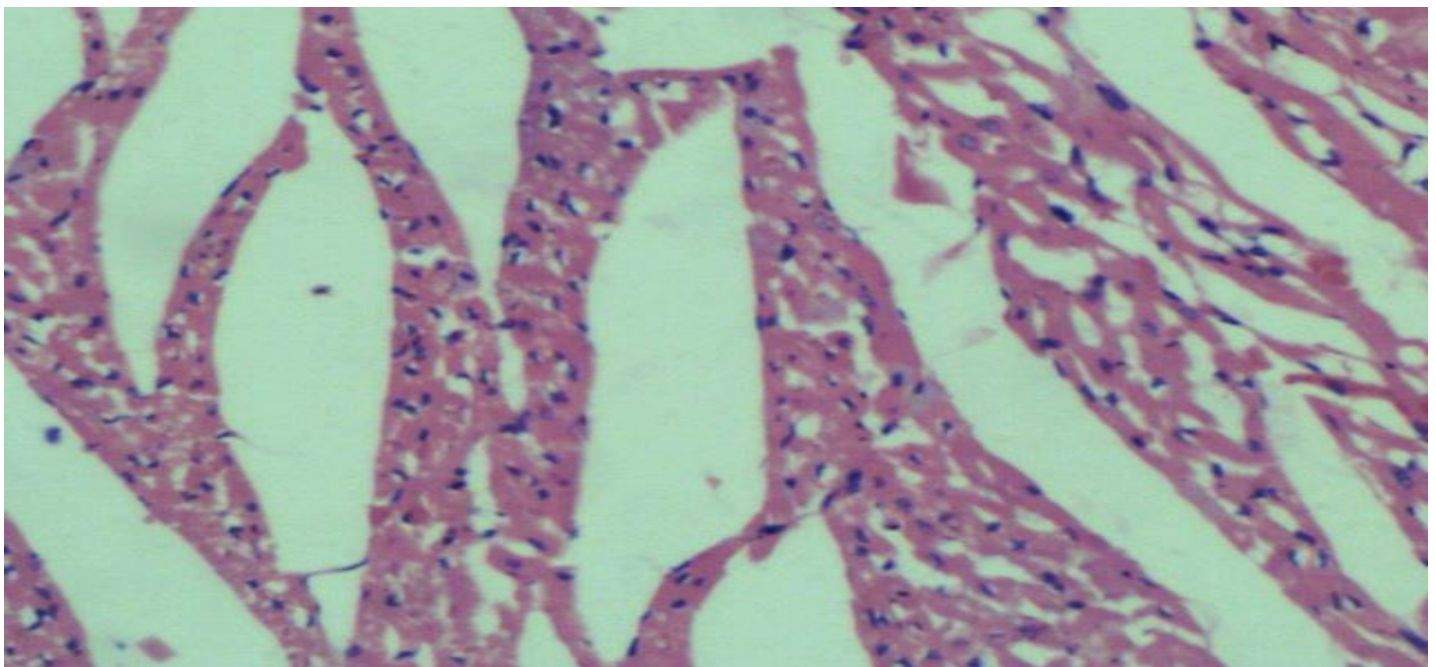
Table3: the *in-vivo* activities of Diminazene aceturate and the plant seed aqueous extract and observed on *Trypanosoma. bruceigambianse* parasite at $10^5/\text{mL}$ in Trypanomiasis disease induced albino rat after 21 days

Group	Group Treatment	Parasite initial level $\pm$ SD of mean at $10^5/\text{mL}$	Day 3 $\pm$ SD of mean	Day 6 $\pm$ SD of Mean	Day 9 $\pm$ SD of Mean	Day 12 $\pm$ SD of Mean	Day 15 $\pm$ SD of Mean	Day 18 $\pm$ SD of mean	Day 21 $\pm$ SD of Mean
A	10 ml of distilled water	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
Normal control		0	0	0			0	0	0
B	200mg of the extract	5.0 $\pm$ 0.0	5.0 $\pm$ 0.0	7.0 $\pm$ 0.0	6.0 $\pm$ 0.00	4.0 $\pm$ 0.00	3.0 $\pm$ 0.0	2.0 $\pm$ 0.0	2.0 $\pm$ 0.0
		0	0	0			0	0	0
C	400mg of the extract	5.0 $\pm$ 0.0	5.0 $\pm$ 0.0	7.0 $\pm$ 0.0	5.0 $\pm$ 0.00	4.0 $\pm$ 0.00	3.0 $\pm$ 0.0	2.0 $\pm$ 0.0	1.0 $\pm$ 0.0
		0	0	0			0	0	0
D	600mg of the extract	5.0 $\pm$ 0.0	5.0 $\pm$ 0.0	7.0 $\pm$ 0.0	6.0 $\pm$ 0.00	4.0 $\pm$ 0.00	3.0 $\pm$ 0.0	2.0 $\pm$ 0.0	0.0 $\pm$ 0.0
		0	0	0			0	0	0
E	800mg of the extract	5.0 $\pm$ 0.0	5.0 $\pm$ 0.0	4.0 $\pm$ 0.0	3.0 $\pm$ 0.00	3.0 $\pm$ 0.00	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0	0.0 $\pm$ 0.0
		0	0	0			0	0	0
F	1000mg of the extract	5.0 $\pm$ 0.0	5.0 $\pm$ 0.0	3.0 $\pm$ 0.0	2.50 $\pm$ 0.0	2.0 $\pm$ 0.00	1.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
		0	0	0	0		0	0	0
G	1200mg of the extract	5.0 $\pm$ 0.0	4.0 $\pm$ 0.0	3.0 $\pm$ 0.0	2.0 $\pm$ 0.00	1.0 $\pm$ 0.00	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
		0	0	0			0	0	0
H	1400mg of the extract	5.0 $\pm$ 0.0	4.0 $\pm$ 0.0	2.0 $\pm$ 0.0	1.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
		0	0	0			0	0	0
I Standard control	3.5mg/kg/bw of Diminazene aceturate	5.0 $\pm$ 0.0	2.0 $\pm$ 0.0	1.0 $\pm$ 0.0	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
		0	0	0			0	0	0
J	10ml of Dextrose saline 10%	5.0 $\pm$ 0.0	6.0 $\pm$ 0.0	8.0 $\pm$ 0.0	10.0 $\pm$ 0.0	20.0 $\pm$ 0.0	Death	Death	Death
Pathologic al control treatment		0	0	0	0	0			

Table 4: The effect of Diminizene aceturate and the plant aqueous seed extract and on the Lipid Profile in mg/dL of post infection treatment in rat

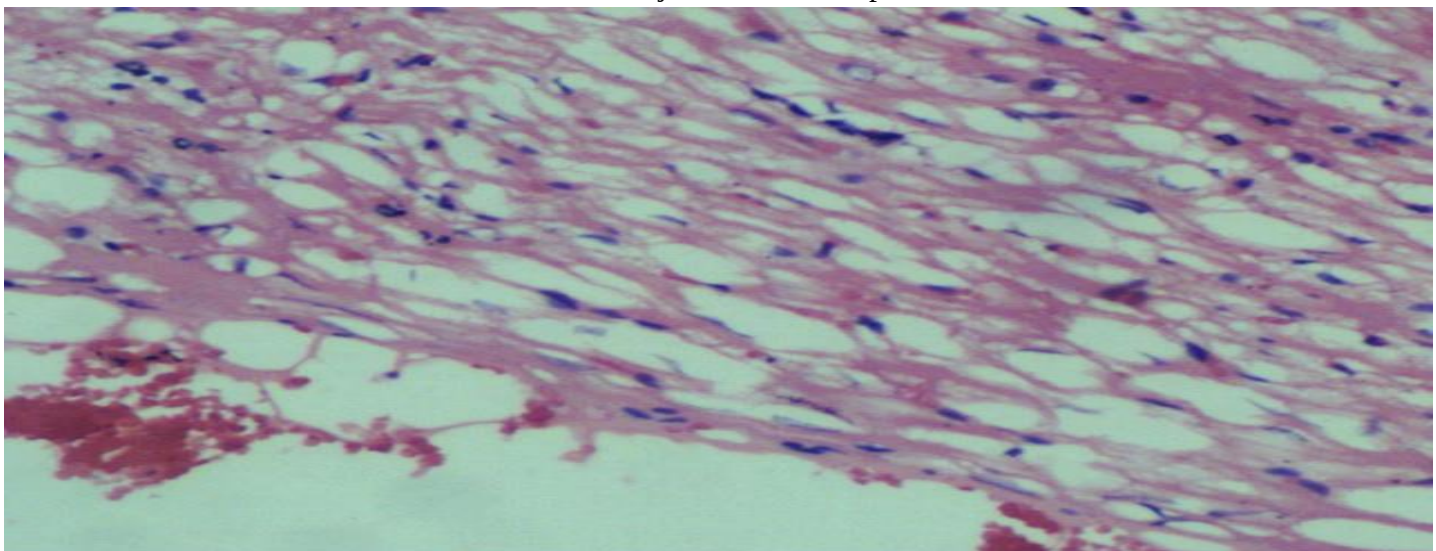
Group	Group Treatment	TC ± SD of Mean	TAG ± SD of Mean	HDL ± SD of Mean	LDL ± SD of Mean
A (normal control)	10 ml of a distilled water	276.50±2.08 ^a	157.25±1.50 ^a	146.75±2.75 ^a	66.00±1.83 ^a
B	200mg/kg/bw of the extract	255.50±1.29 ^c	155.50±1.91 ^{ab}	143.75±1.71 ^a	63.75±1.71 ^a
C	400mg/kg/bw of the 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 the 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 the 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 the 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 the 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 the extract	200.75±0.96 ^h	119.00±0.82 ^f	116.50±1.91 ^f	48.00±0.82 ^d
I (Standard drug control)	3.5mg/kg/bw of drug	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 10% Dextrosaline solution	121.50±1.29 ⁱ	104.50±1.29 ^g	129.25±1.89 ^{cd}	58.25±0.96 ^{bc}

Note: letters a,b,c, d... superscripts on mean ± S.D denotes the significant differences of the means at $p < 0.01$.
 Key: TC (Total cholesterol), TAG (Triglyceride), HDL (High density lipoprotein), LDL (Low density lipoprotein)



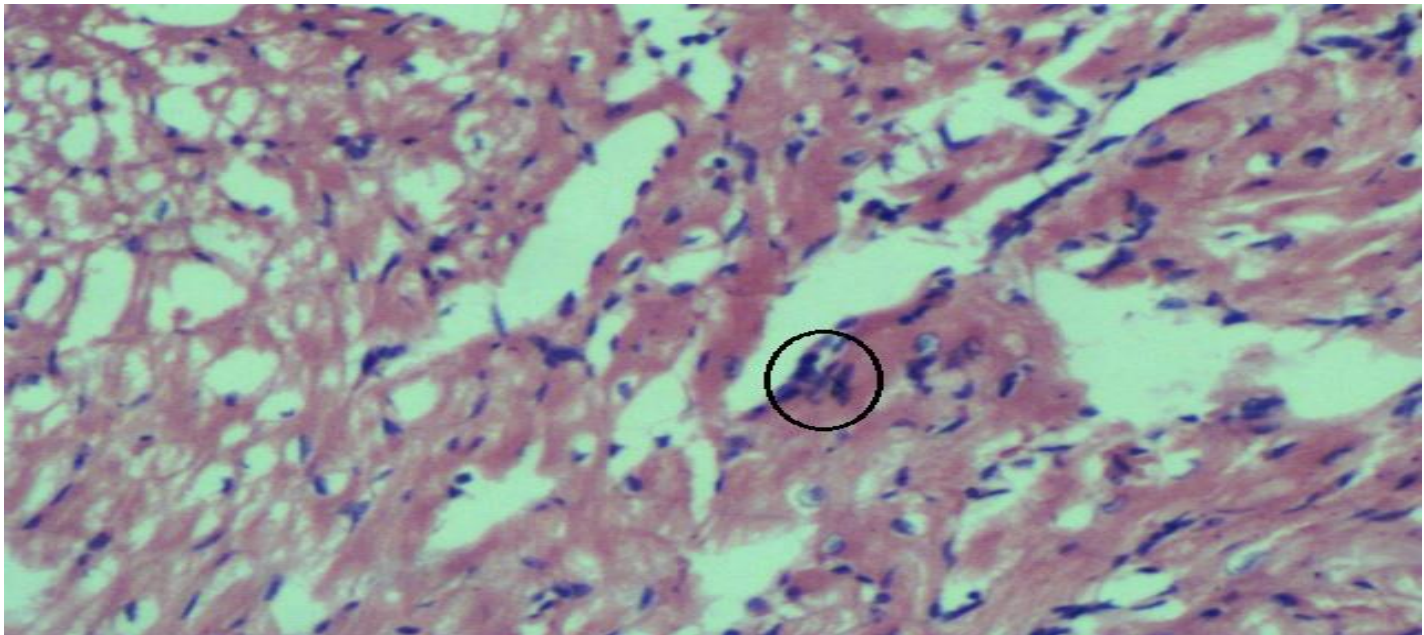
AH

Plate 1: Group Anormal control. **Heart** tissue revealing a normal architecture after stained with Heamatoxillin&Eosin stain and viewed at x 100 objective microscope lens,



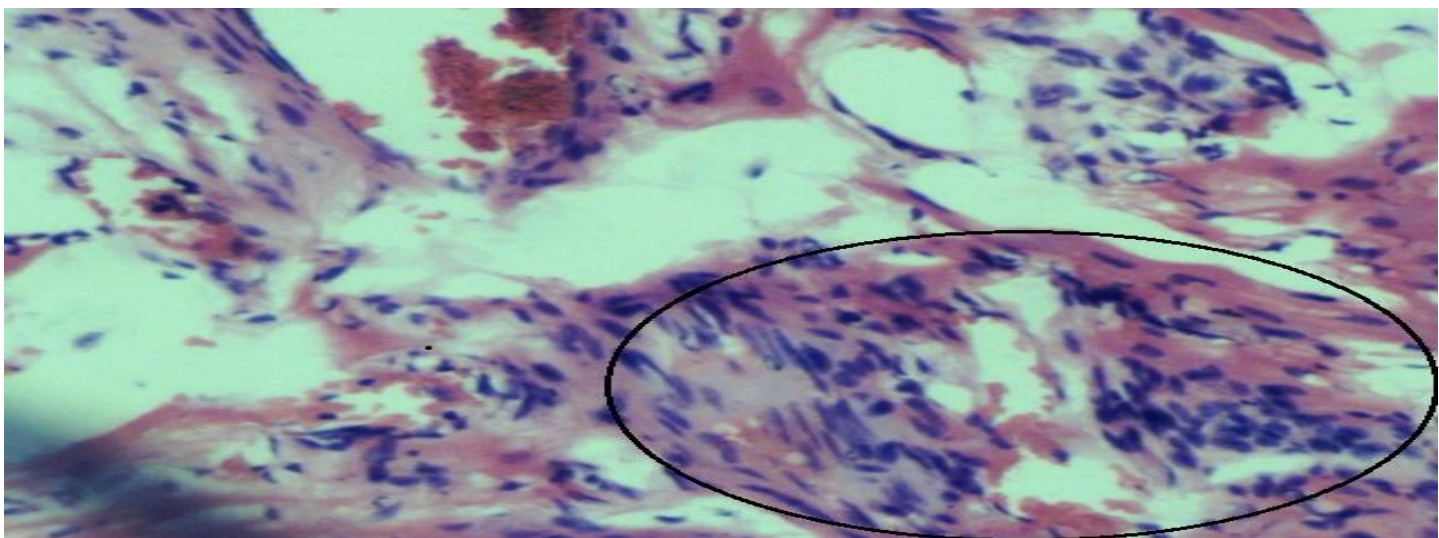
BH

Plate 2: The effect of 200 mg of the plant extract on the **heart** of Group B which indicated no clear degenerative changes architecture after stained with Heamatoxillin&Eosin stain and viewed at x 100 objective microscope lens,



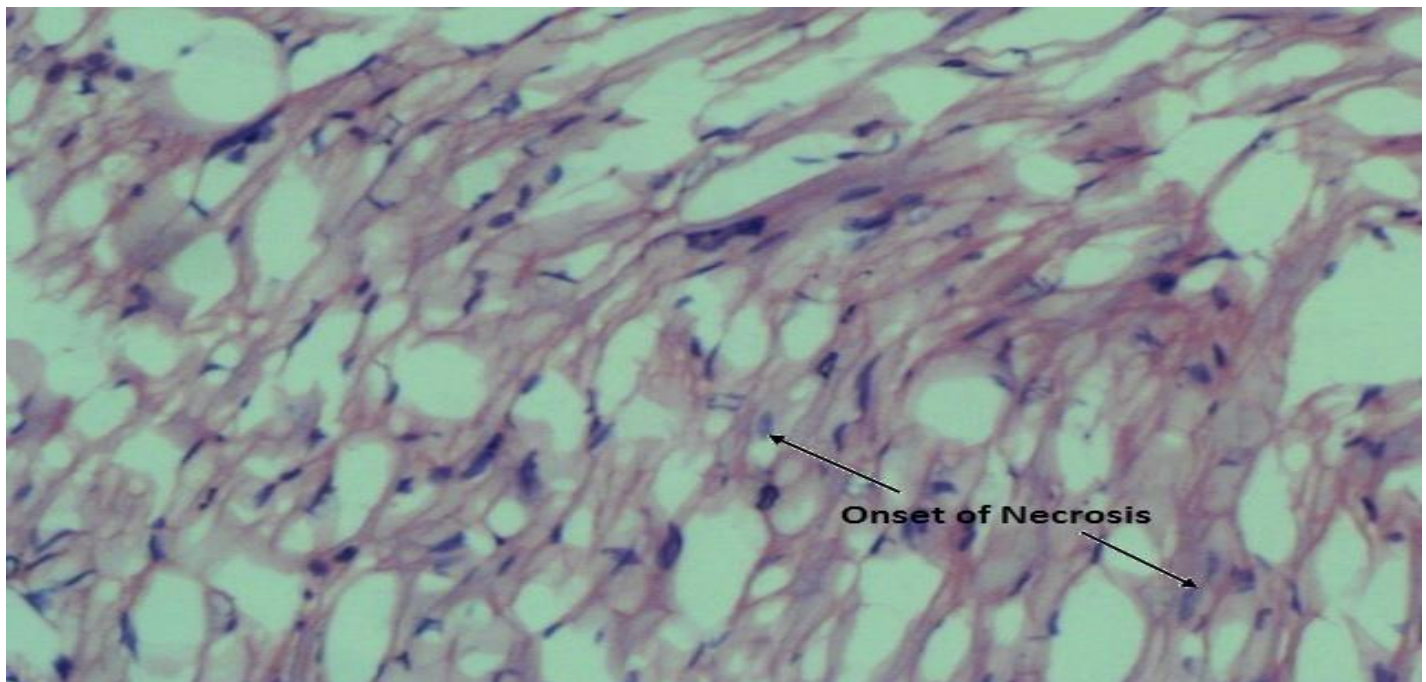
CH

Plate 3: The effect of 400 mg of the plant extract on the **heart** of Group C which indicated moderate inflammation after stained with Heamatoxillin&Eosin



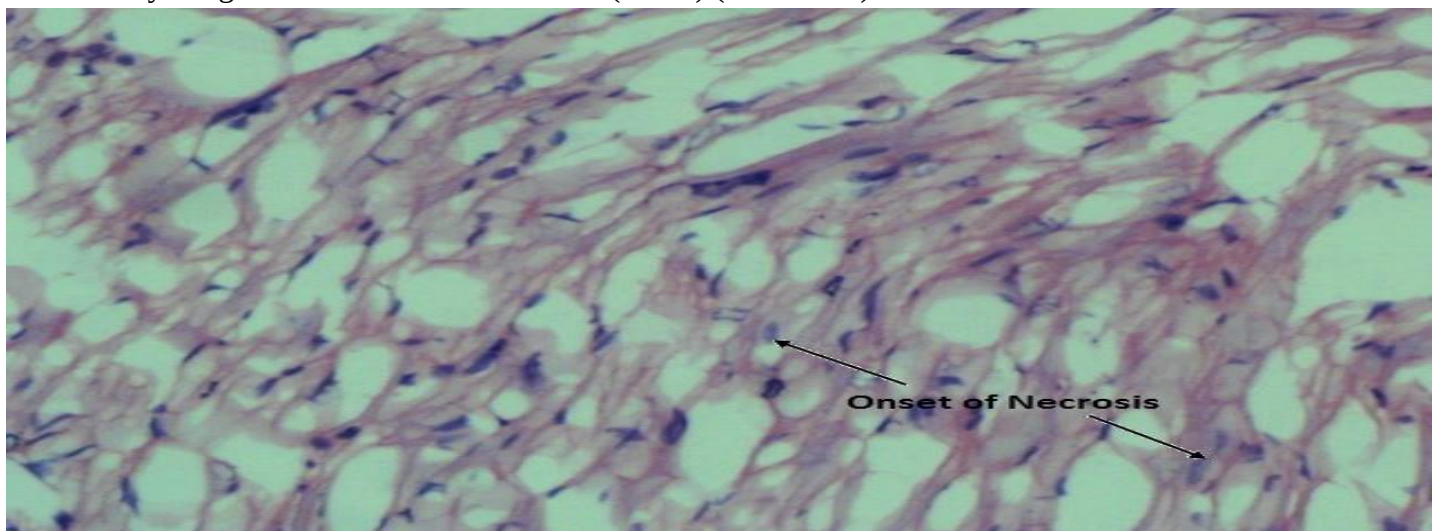
DHP

Plate 4: The effect of the plant extraction the **heart** of Group D indicated by an acute inflammatory infiltrate of the heart with the circle after stained with Heamatoxillin&Eosin stain then viewed at x 100 objective power microscope lens



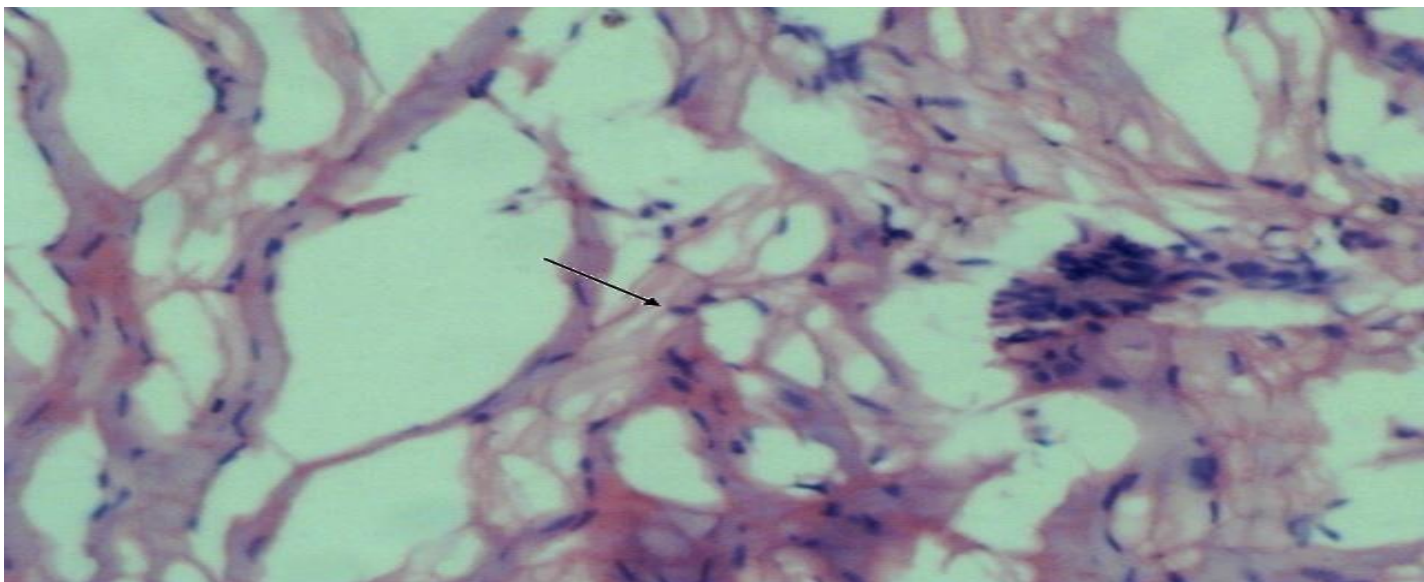
EH

Plate 5: The effect of 800mg /of the plant seed aqueous extract on the **heart** of Group E (Infected and treated) indicated by the gradual onset of necrosis with (arrow) (H&E stain) x 100



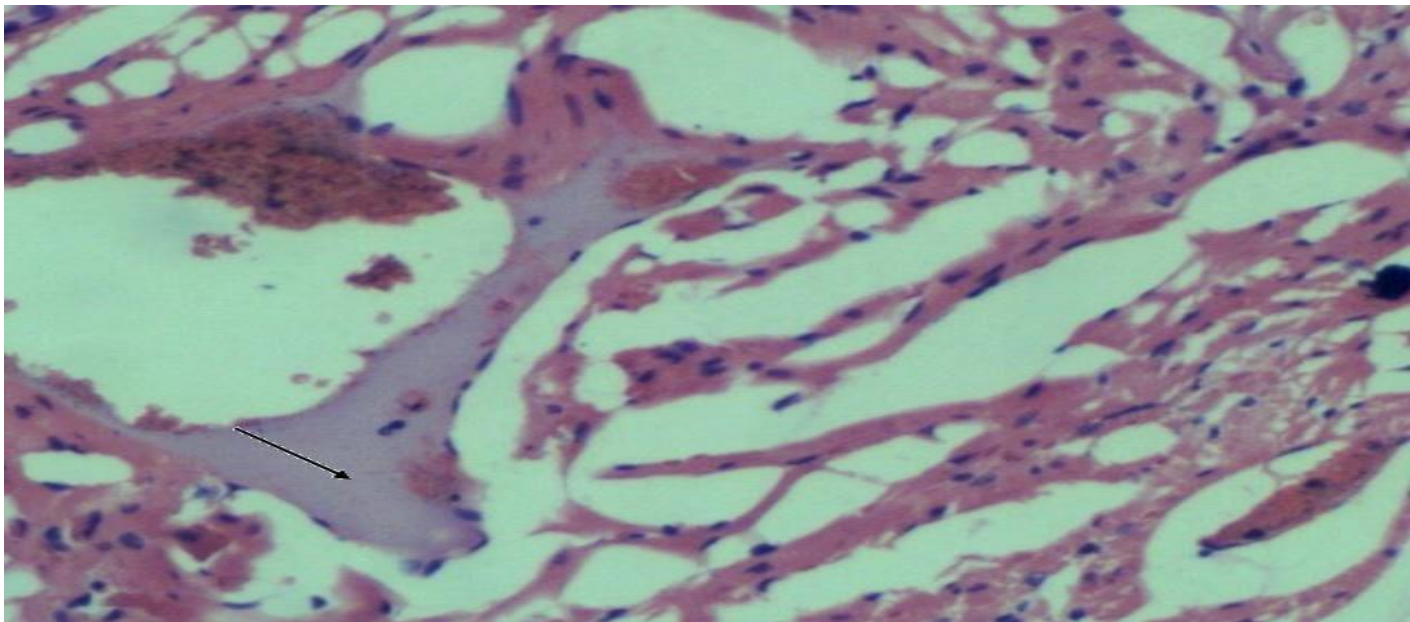
EH

Plate 5 The effect of the 800mg of the plant aqueous seed extract on the **heart** of Group E indicated by the gradual onset of necrosis with an indication (arrow) after staining with Heamatoxillin and eosin stain and viewed at x100 objective microscope lens



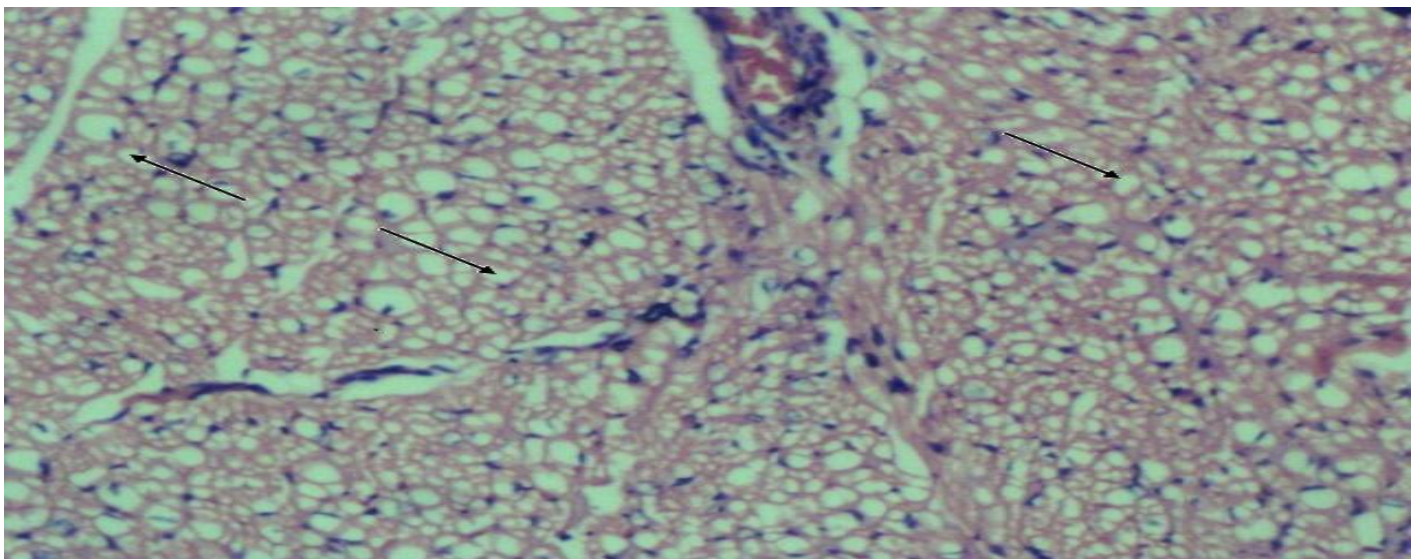
FH

Plate 6: the effect 1000mg of the plant seed extract on the **heart** of Group F Indicated by the myocardial fiber in disarray with an Arrow indication (Heamatoxillin&Eosin stain then viewed atx 100 objective microscope lens,



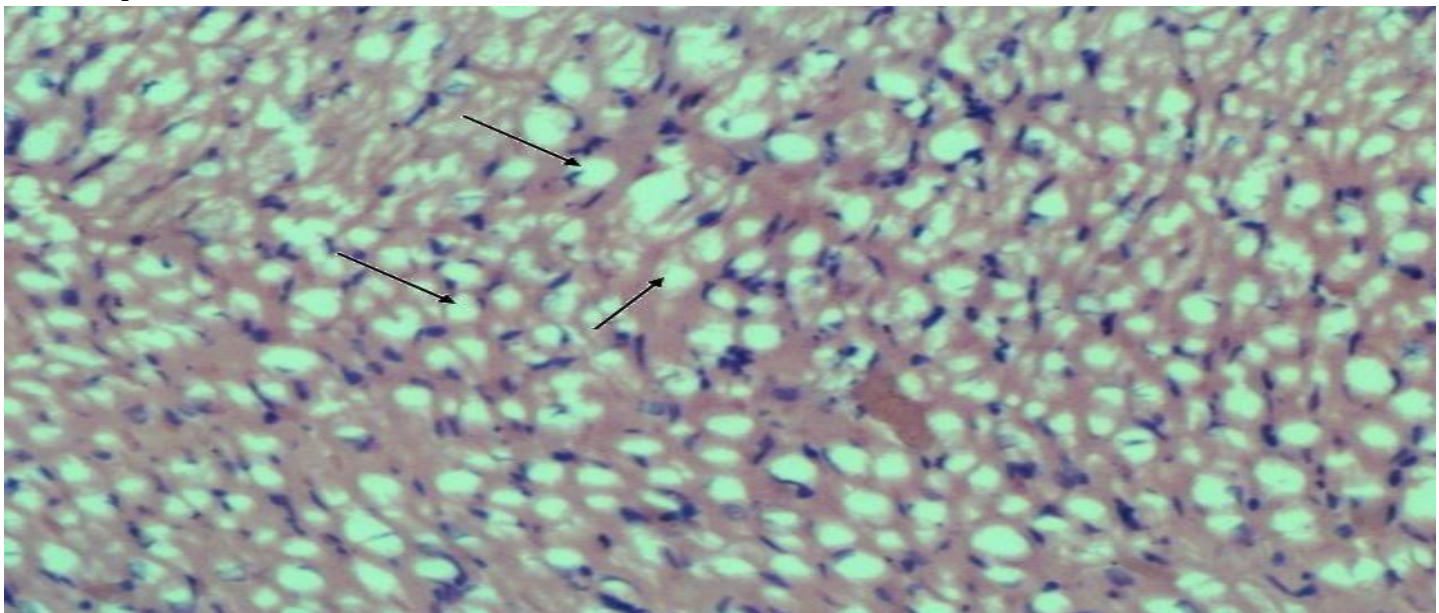
GH

Plate 7: Effect of 1200mgof the plant seed extract on the **heart** of Group G Indicated with the evidence of hyaline deposited with an (Arrow) Heamatoxillin&E stain then viewed at x 100 objective microscope lens



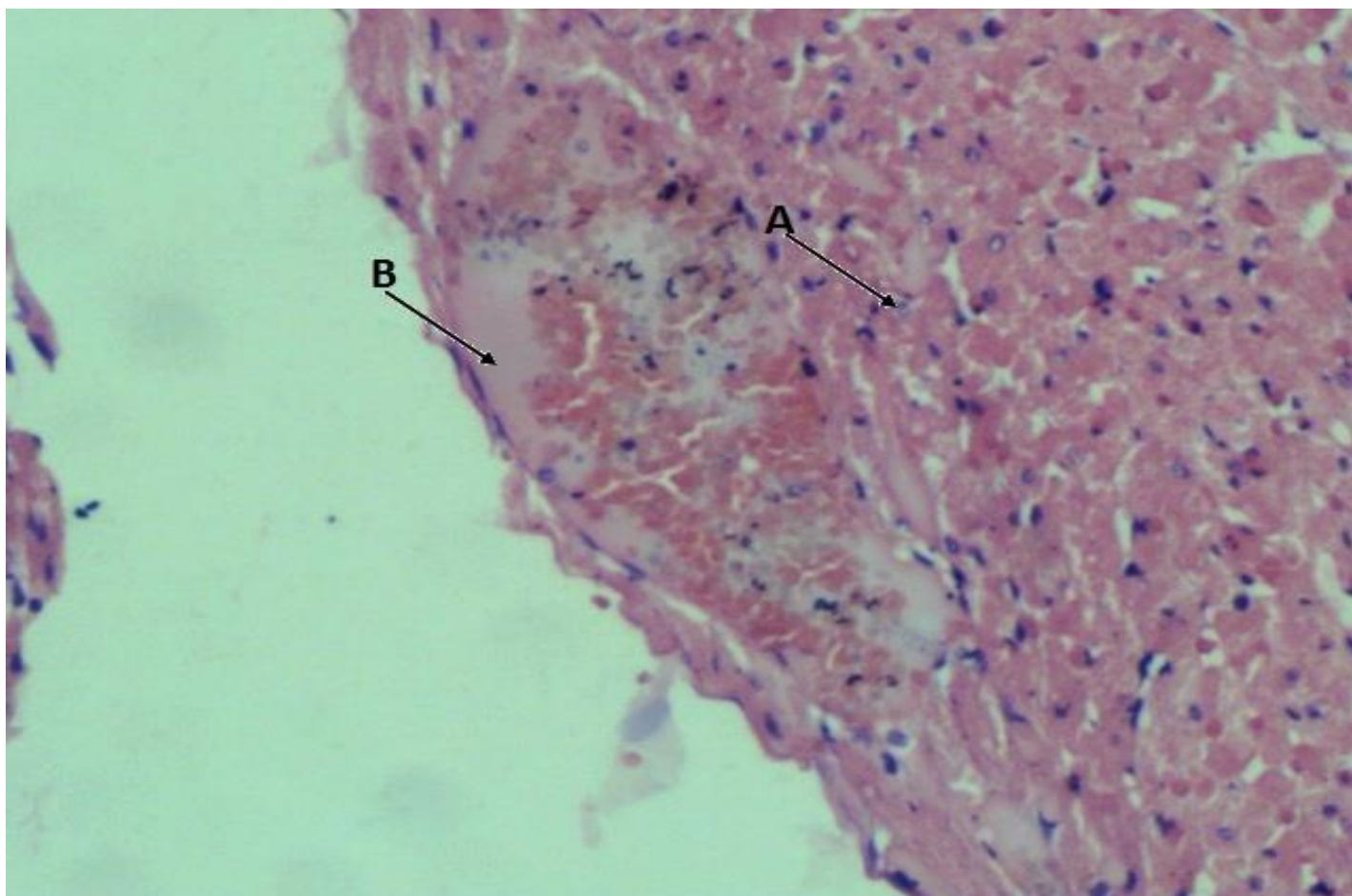
HH

Plate 8: Shows the effect of 1400mg of the plant seed extract on the **heart** of Group H indicated by the evidence of fatty change (Steatosis) with the Arrow Heamatoxillin&Eosin stain then viewed at x 100 objective microscope lens



IH

Plate 9: The effect of Diaminizen eaceturate (Diminizen eaceturate) standard drug on the **heart** of Group I indicated by the evidence of fatty change (Steatosis) with an (Arrow)Heamatoxillin& Eosin stain then viewed at x100 objective microscope lens



JH

Plate 10: Effect of parasite on **heart** of Group J pathological control with an evidence of necrotic change A and hyaline deposition B with arrow Hematoxylin & Eosins stain then viewed at x 100 objective microscope lens.

Discussion of results.

The Bitter kola seed extract has been widely used in traditional medicine in African and Asia for various medicinal purposes, this present study evaluated the photochemical composition, cytotoxicity of the heart, effect on lipid profile and Anti-trypanosomal effects of Bitter kola aqueous seed extract and Diaminazene aceturate drug in Trypanosomiasis disease induced Albino Wistar rat, the phytochemical screening of the plant seed extract revealed the presence of Saponin, Alkaloids, Taninins, Anthraquinones, Glucoside, Flavoids, Carbohydrate, Terpenes, Steroids and Quinones, from the photochemical screening Steroid has the highest concentration $36.13 \pm 1.00/100g$ and Anthraquinones, has the lowest $1.4 \pm 0.01/100g$. This result compared well with the outcome of the study of (Abiodun, *et al.* 2021) who reported that *Garcinia kola* seed contained alkaloid, 2.3 ± 0.05 , saponine, 2.42 ± 0.04 , flavonoids, 2.05 ± 0.03 , steroid, 31.13 ± 1.00 respectively in 100g of

powdered *Garcinia kola* seed extract. Findings from this study indicated the presence of those photochemical may have contributed to the trypanocidal activity and its effect observed in the study when compared with Bereni standard drug. Different concentrations of the plant seed aqueous extract were studied for their *in-vitro* and *in-vivo* anti trypanosomal activities against *T. bruceigambianse* parasite and compared with Bereni drug. Decrease in the population of motile trypanosome when compared with standard drug was taken as measure of *in-vitro* trypanocidal activities of the extract. From the results in Table 2, it was also indicated that the seed extract at 1400mg has the highest *in-vitro* activity by reducing the parasite population from 5.00×10^5 to 0.00×10^5 within the 6 hours of post incubation period and the 200mg produced the least effect in reducing the periodic population from 5.0×10^5 /ml to 4.0×10^5 /ml with in the first Twenty four hours post incubation time with this observation. it was shows that the plant extract activities was similar to that of Diaminizene aceturate the standard trypanocidal drug served as control during this study with a well-established mechanism of action. (Ekesiobi, *et al.* 2024, Abiodun, *et al.*, 2024.). The lowest activates of the extract was observed *In-vitro* in 3 hours - 24 hours at a low concentration of 200, 400, and 600mg. this may be as a result of the lower concentration of active photochemical component of the plant seed. This result equate with the former result of the study carried out by (Abiodun *et al.* 2018) who indicated that extract of *Moringaolifera* leaves and back had a little significant effect on the parasite during a 2 hour of incubation this variation may be contributed to different types and concentration of the photochemical components in the various parts of the plant. When the *Garcinia. kola* seed extract concentration was tested on the parasite for *in-vivo* activities it was shown in Table 3 with the reduction in parasite load in all the extract concentration treated groups, 200 - 1400 such that 200 mg of the extract reduced the parasitic load from $5.0 \times 10^5 \pm 0.00$ to $2.0 \times 10^5 \pm 0.00 \pm SD$ of mean within 21 days which save as the lowest concentration that produced trypanosotatic effect on the parasite, and the 1400 mg indicated the highest effectiveness of the extract by reducing the parasitic population load from $5.00 \times 10^5 \pm 0.00$ to $0.0 \times 10^5 \pm 0.00$ in 12 days of post parasitic infection treatment of the rats this save similar to the trypanocidal effect of 3.5 mg drug from $5.00 \times 10^5 \pm 0.00$ to $0.0 \times 10^5 \pm 0.00$ in 3days post- animals infection treatment when its compared with the pathological control that the parasitemia increased geometrically from $5.00 \times 10^5 \pm 0.00 - 20.00 \times 10^5 \pm 0.00$ within 12 days of post-parasiteanimal infection treatment till the death of the some rats were observed due to parasite effects from day 15-21 at ($p < 0.01$). This significant observation of trypanocidal effect resemble the formal report of (Abiodun *et al.* 2021) that signified *Terminaliaavicenniodis* plant leave extract significantly suppression and total clearance of the parasite in 14 days Post infection The same trend of result also noticed on triglyceride and high and low density Lipo- protein of all the infected and extract treated groups, standard groups and pathological group and also in Total cholesterol. There was a similarity of this result with that of (Abiodun, *et al.* 2024) who observed that the total Cholesterol (TC), Low density lipoprotein, High density lipoprotein and Triglyceride (TAD) level decreased significantly at ($p < 0.05$) with Trypanosoma. *Brucei* brucei infected Wister rat when justaponized with the normal healthy rat but increased drastically at ($p < 0.05$) when the rats was treated with the standard drug and the extract. Therefore,

with this report it was an indication that a lipid plays an important role in the pathogenesis of trypanosomiasis disease (Abiodun, *et al.*, 2021, Abiodun, *et al.*, 2018;) Therefore from the results findings of this study it can be deduced that *T. brucei gambiense* parasite infection can cause a significant decrease in the serum level of Total cholesterol, LDL HDL, and TAG, which is also similar to the other report made by (Abiodun, *et al.* 2024) though it's very difficult to ascertain the reason behind the reason why there was a sudden fall may be there is a pathological mechanism that likely to be involved The study of the effect of Diaminazene aceturate and aqueous seed extract of *Garcinia kola* on the Heart of trypanosomiasis disease induced rat indicated by many pathological lesions as result showed in Plates 1- 10 with an indication of Normal renal architecture, of the heart of group A Normal control and Also some heart histopathological effects were observed on the heart of all the extract treated group except in 200mg of the extract doses from 400-1400mg, and standard Diaminazene aceturate drug control at 3.5mg.

Conclusion

In conclusion from the results this study it was revealed that *Bitter kola* aqueous seed extract has some photochemical materials that can produce a negative effect on *Trypanosoma brucei gambiense* parasite proved to be trypanostatics at low a concentration of 200mg and trypanocidal at concentration higher than 400mg *in-vivo* and *in-vitro*, the seed extract also showed low side effect on the Biochemical index of Wister rat at concentration less than 400mg and produced severe effect on both parameters at higher concentration greater than 400mg. Also, *Bitter kola* seed extract also exhibited highly toxic to the Heart at high concentration greater than 200mg as it was compared with Diminazene aceturate which also exhibited similar level of typanosomal activity and high cytotoxic effect on the heart tissue at standard recommended dosage of 3.5mg

Recommendations

Based on the result of this current study, *Garcinia kola* seed can be serve as novel compound in the search for the production of new affordable and more effective anti trypanosomal therapy as a replacement to Diminazene aceturate in treatment of trypanosomiasis disease in both human and animal due to the presence of many active photochemical compound it possess,.

References

- Abiodun, M.O. Ekesiobi, A.O., Leonard, C.O. (2024). Exploring the in-vitro and in-vivo Trypanosomal Activities of *Garcinia kola* (Bitter kola) Seed Aqueous Extract Using Animal Models. ABUAD International Journal of Natural and Applied Science. **4** (2): 113-120. ISSN: 2955-1021, <http://doi.org/10.53982/aijnas.2024.0402.14>
- Abiodun, M.O., Ekesiobi, A.O., Leonard, C.O. (2024). Ant itrypanosomal Activities and Cytotoxic Effects of *Garcinia kola* (Bitter kola) Aqueous Extract and Berenil in Abino Wister Rats. Journal of Pharmaceutical and Applied Science. **21** (4): 4326-4349. ISSN: 1596- 8499. www.ajol.info/journal/jophas

- Abiodun, M.O., Ekesiobi, A.O., Leonard, C.O.(2024). Anti-trypanosomal efficacy of *Garcinia kola* aqueous seed Extract as an Alternative remedy in treatment of trypanosomiasis disease. International journal of Medical Science and Applied Bioscience **9**(1): ISSN2545-5877.<http://www.casirmediapublishing.com>.
- Abiodun, M.O., Ekesiobi, A.O., Leonard, C.O.(2024): Anti-trypanosoma activities, Histological and Kidney Function Effect of *Garcinia kola* seed extract and Standard drug (DiaminizeneAceturate) in trypanosomiasis disease induced albino Wister rat. Adeleke University Journal of Science **3**((1): 238-299. ISSN:2814-0427. <https://aujs.adelekeuniversity.edu.ng>
- .Ekesiobi, A.O, Abiodun, M.O., Leonard, C.O. (2024). Pharmacological potential and effect of Bitter kola aqueous seed extract compared with the standard drug in trypanosomiasis disease induced animal. Achievers Journal of Scientific Research. **6**(2):34-49. Doi: 10.5281/ zenodo.14566210. www.achieversjournalofscience.org.
- Abiodun, M.O., Abiodun, I.K., Ekesiobi, A.O and Onyenwife, L.C.(2021). Anti-trypanosomal Activities, Liver enzymes and Histological effect of *Garcinia kola* Aqueous Seed Extract and Berenil (DiaminizeneAceturate) drug in Trypanosomiasis induced albino Wister Rat. *International Journal of innovativescience research technology*. **6** (11):331. ISSN No: 2456-2165. www.ijirst.com
- Abiodun, M.O., Abiodun, I.K., Ekesiobi, A.O and Onyenwife, L.C.(2021). Anti- trypanosomal activities and effect of *Garcinia kola* aqueous seed extract in Trypanosomiasis Induced Wister rat. *International Journal of innovative scienceand research technology*. **6**(11): 330
- Abiodun, M.O., Abiodun, I.K., Ekesiobi, A.O and Onyenwife, L.C.(2021). Anti-trypanosomal Activities, Liver enzymes and Histological effect of *Garcinia kola* Aqueous Seed Extract and Berenil (DiaminizeneAceturate) drug in Trypanosomiasis induced albino Wister Rat. *International Journal of innovativescience research technology*. **6** (11):331. ISSN No: 2456-2165. www.ijirst.com
- Abiodun, M.O., Abiodun I. k. and Kalu, M.U (2018). Antifungal Effectiveness of Zingiber Officianale Plant Root Extract on *Aspegillus niger* and *Rhizopusstolonifer* Compared with Standard Drug. *International and Journal of innovative science and research technology* **3**(11); 359-363. ISSN No: 2456-2165. www.ijirst.com
- .Adeyemi, O.S., Akanji, M.A. and Ekanem, J.T.(2012). Ethanolic Extract of *Psidiumguajava* Influences Protein and Bilirubin Level in *Trypanosome bruceibrucei* infected Rat. *Journal of Biological Sciences*, **12**: 111 – 116

- Emmanuel, O.O., Adamu, Y.K. and Roseline, F.O. (2011). Preliminary Studies of the Antitrypanosomal Activity of *Garcinia kola* Nut Extract in Mice Infected with *Trypanosome brucei*. *Journal of Medicine and Medical Science*, **2**(1): 628 – 631.
- Evans, W.C. (1996). Trease and Evans Pharmacology. W.B. Saunders, University of Michigan, United States of America, pp 67 -76.
- Igoli, J.O. Gray, A.I., Clements, C.J. and Moued, H.A. (2011). Anti trypanosomal Activity and Cytotoxicity of Some Compound and Extract from Nigeria Medicinal Plant in Phytochemicals. *Bioactivity and Impact on Health Rasooli*, **5**: 345-358.
- Lutje, V., Seixas, J. and Kennedy, A. (2013). Chemotherapy for Second Stage Human Africa Trypanosomiasis.
- .Omwirhimen, E.M., Aseton, O.A. and James, S.A. (2017). The Photochemical Constituents and Relatives Antimicrobial Activities against Clinical Pathogens of Different Seed extract of *Colanitida* (Heckel) grown in South West, Nigeria. *Journal of Pharmacology and Phytochemistry*, **6**(1): 493 – 501.
- WHO (2005). Mortality and Health Data Statistic. Accessed 10 February, 2005.