Effects of *Sida corymbosa* Leaf-Extract on the Lipid Profile of Carbon Tetrachloride-Injected Male Rats

ABSTRACT

Aim: This study aimed to investigate how *Sida corymbosa* ethanolic leaf extract affected the lipid profile of rats injected with carbon tetrachloride (CCl_4).
Study design: Rats used were grouped into 32. Groups A1-A4: Negative control (those given feed and water only), B1-B4: Positive control (those that stayed for 28 d + CCl_4only), C1-H4: Treatment groups (those given *Sida corymbosa* extract at 100, 250 and 500 mg/kgBW before and after injecting CCl_4).
Place and Duration of Study: This study took place at the Department of Human Biochemistry Laboratory, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University (NAU), Nnewi, Anambra State, Nigeria, between December 2022 and January, 2023.
Methodology: A total of 160 Albino-Wistar male rats which were about 12 weeks old (170-180 g) were used in the study. Lipid profile assays (serum total cholesterol-TCHOL, triglycerides-TRIGS, high-density ~~lipoprotein(~~lipoprotein (HDL), and low-density lipoprotein (LDL) were determined in this study. Results were subjected to statistical analysis using SPPS statistical software, version 25.
Results: For rats administered CCl_4 before administration of *Sida corymbosa* ethanolic-leaf extract at 500, 250, and 100 mg/kgBW from 7 to 28 d (treatment groups-C1-E4), serum TCHOL decreased significantly (P< 0.05) in treatment groups (131.69+/-16.08-94.48+/- 18.41, 150.26+/-0.06-109.67+/-0.67,272.97+/- 66.26-151.09+/-17.56) when compared with the untreated groups-B1-B4 (279.22+/-55.25-398.90+/- 0.58) and negative control Groups-A1-A4 (87.92+/-3.40-88.58+/-16.14. High density lipoprotein increased significantly (P< 0.05) in treatment groups (58.96+/-2.18-65.99+/-1.04~~,46.73~~, 46.73+/-1.41-61.23+/- 16.29, 64.73+/-3.05-76.41+/-1.18). Triglyceride, and LDL also decreased significantly (P< 0.05) among the treatment groups when compared with the positive control (B1-B4) (313.47+/-5.69-388.46+/-0.5053, 127.36+/- 53.97-215.38+/-0.66) and negative control groups (67.04+/-15.48-66.75+/-16.75, 50.01+/-14.66-50.00+/-15.02). **Conclusion**: *Sida corymbosa* ethanolic-leaf extract, therefore, have protective and curative effects against rats induced hyperlipidemia with CCl_4 in a dose-dependent manner.
.

Keywords: Albino Rrats, Carbon tTetrachloride, Lipid pProfile aAssay, Risk fFactors, Sida pPlants,

1. INTRODUCTION

Lipid profiles have been known to include total cholesterol (TCHOL), apolipoprotein, triglycerides (TRIGS), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Elevated levels of total cholesterol, triglycerides, low-density lipoprotein, and apolipoproteins (hyperlipidemia) have been reported to be risk factors for cardiovascular diseases [1-3]], and other heart-related diseases. The increase of cardiovascular illnesses globally is alarmingly high as a result of the

nature of foods, which many people feed on. A lot of people feed more on fatty foods than vegetables and minerals. The worldwide prevalence of hyperlipidemia has been alarming. The projection is that it will continue to increase in the future [4]. Cardiovascular disease (CVD) has been reported to be among the major causes of death all over the world [5]. As a result, coronary artery disease (CAD) is among the diseases linked with elevated levels of lipids, as this happends when lipid accumulates in the walls of the arteries [6].

Diseases such as pancreatitis have been associated with increased levels of triglyceride [7]. An abnormal lipid profile can be discovered through routine lipid diagnosis by collecting blood samples intravenously and analyzing them using spectrophotometer using appropriate reagent kits. An abnormal lipid profile has also been reported to be linked with diabetes [4].

According to Okonkwo [8], Dike [9-10], the use of medicinal plants in the treatment of diseases has been ongoing in various parts of Africa, including Nigeria and some parts of Asia. There are many trees and shrubs in Nigeria with many medicinal and nutritional values (Okonkwo [8]. Plants have been used as a basic source of raw materials in the production of synthetic drugs and their allied products. Traditional medical practice has been taking place in African countries long before the use of modern drugs and modern medical practices. A lot of plants have antioxidant properties and can be used as traditional medicine for managing stress, aging, and age-related diseases such as cardiovascular diseases [11]

Sida corymbosa plants have been known in many parts of Africa to be used in treating diseases such as ulcers, liver problems, infertility, and dysentery [12-13]. Sida plants have over 200 species and many subspecies, among which is *Sida corymbosa*. *Sida corymbosa* is one of the sida plant species found in various parts of Eastern, Western, and Northern Nigeria, where people claim to be using it to treat a variety of diseases, including abnormal lipid *profiles.* Every part of this plant, including bark, root, seed, and flower, is used in traditional medicine [14]. It is known to be a stubborn species, surviving in all weather and all modes of propagation. The scope of this work covered assay on lipid profile of rats given CCl_4 and treated with *Sida corymbosa* extract which include; serum total cholesterol, high density lipoprotein, low density lipoprotein and triglycerides. There has been a lot of research done on the use of *Sida corymbosa* extract in treating diseases like liver, bleeding, and infertility, but not on its use in treating abnormal lipid profiles. This work is therefore justified by closing this gap. This study is aimed at investigating the effects of *Sida corymbosa* ethanolic-leaf extract on the serum lipid profile of Male-Albino Wistar rats injected with carbon tetrachloride (CCl_4). This work also looked at dosage effects and duration of the extract on the animals.

Figure 1: A photograph of the *Sida corymbosa* Pplant

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1. Materials

The chemicals that were used in this work were of analytical grade and were obtained from the British Drug House (BDH) Ltd., Poole, England, through their sales representative in Ikeja, Lagos State, Nigeria. Materials used include diethyl ether, absolute ethanol (98%), lipid profile reagent kits, animal cages, sterile syringes, capillary tubes, plain specimen bottles,

centrifuge machines (Model 800D, China), rotary evaporator (Model TT22, USA), oven, and Ultraviolet-Visible Spectrophotometer (Model 752N, China). All reagent kits were obtained from Randox Laboratories Ltd., UK, via their sales representative in Ikeja, Lagos State, Nigeria. Distilled water used was obtained from the Department of Human Biochemistry Research Laboratory, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. The albino rats used were obtained from the Animal Facility Unit of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. *Sida corymbosa* plants were obtained from Otolo-Nnewi in the Nnewi-North Local Government Areas of Anambra State. They were identified and authenticated at the Department of Botany, Nnamdi Azikiwe University-Awka, Anambra State, Nigeria.

2.2. Methods

2.2.1. Preparation of Plant Materials

The leaves of the plants were prepared according to the method described [15]. The leaves of the plants were washed with distilled water, dried under air at room temperature, and ground using a blender (Model 989Z, made in China). The ethanol extract was obtained by soaking 25 g of dried leaves in round bottom flasks containing 200 ml of absolute ethanol (98 %) for forty-eight hours with ocassional shaking. The ethanol extract was filtered using 40 mm Whatman filter paper and was evaporated using rotary evaporator (Model: TT22, USA) at 65 ^{O}C. The extract was dried in an oven (Model: TT-9023A, China) at 44 ^{O}C. The samples were stored in a refrigerator (Model: GR-B25VPL, India) at +2 ^{O}C for further analysis.

2.2.2. Acute Oral Toxicity Test (LD_{50})

The LD_{50} of the ethanol-leaf extract of *Sida corymbosa* plant has been determined by [12], to be above 5000 mg/kgBW and confirmed according to [16] by administering 5, 000 mg/kgbw of the ethanol extract to each of two groups of one rat each. The observation was carried out on the rats for 1h after administration and 10 min for every 2 h interval for 24 h.

2.2.3 Animal Handling

Rats obtained from the Animal Facility Unit of College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus, were used in this study. They are fed on a pellet diet gotten from Gland Cereals Ltd., Jos, Plateau State, Nigeria, via her sales representative at Nnewi, Anambra State, Nigeria.

2.2.4. Experimental Design

A total of -160 adult male Albino- Wistar rats of about 12 weeks old, weighing 170-180 g each were used for this study. They were grouped as follows:
Groups A1-A4: Negative control-those given feed and water only for 7-28 d
Groups B1-B4: Positive control- those that stayed for 7-28 d + CCl_4 only.
Groups C1-C4: Those given 500 mg/kgbw of extract for 7- 28 d + CCl_4 (Protective studies)
Groups D1-D4: Groups given 250 mg/kgbw of extract for 7- 28 d + CCl_4 (Protective studies)
Groups E1-E4: Groups given 100 mg/kgbw of extract for 7- 28 d + CCl_4 (Protective studies)
Groups F1-F4: Those given 500 mg/kgbw of extract for 7- 28 d + CCl_4 Curative studies)
Groups G1-G4: Groups given 250 mg/kgbw of extract for 7- 28 d + CCl_4 Curative studies)
Groups H1-H4: Groups given 100 mg/kgbw of extract for 7- 28 d + CCl_4 (Curative studies).

2.2.5. Studies on Protective and Curative Effects of *Sida corymbosa* Ethanolic Leaf Extract ~~Against~~against Abnormal Lipid Profile

A total of 80 Male-Albino Wistar-rats were used for each study. The rats were grouped into 32 groups containing 5 rats each. Twelve groups were administered *Sida corymbosa* ethanol-leaf extract orally using oral canular at 500, 250 and 100 mg/kgbw for 7 d, 14 d, 21 d and 28 d before injecting 0.3 ml/kgbw of carbon tetrachloride at the end of each duration intraperitoneally using olive oil as vehicle in the ratio of 60: 60 v/v. Another 12 groups were administered *Sida corymbosa* ethanol-leaf extract orally using the same method above after injecting CCl_4.

2.2.6. Blood Sample Collection

This was done according to the method described by [17] via orbital sinus (Periorbital or orbital plexus bleeding).
Procedure
The rats were anesthetized using diethyl ether (98.6 %). They were then scruffed with thumb and forefinger of the nondominal hand and the skin around the eye was pulled out. This was followed by inserting a capillary tube into the medial canthus of the eye (30 ^{O}C angle to the nose of the animal). The tissue was punctured and the capillary tube

entered the sinus to puncture it. This was punctured and blood came out. The blood samples were collected using plain specimen bottles and centrifuged at 3000 rpm for 20 min at room temperature using Ultra-Modern Centrifuge Machine (Model 800D, China). The serum was separated and stored in a refrigerator (Model GR-B25VPL, India) at $+2^{O}C$ for onward analysis.

2.2.7. Determination of Serum Lipid Profile

This was done by using UV-VIS Spectrophotometer (Model 752 N, China) as recommended by [.18]

2.2.7.1. Serum total cholesterol (TCHOL)

Principle

Total serum cholesterol was determined on the principle that cholesterol undergoes hydrolysis and oxidation to form quinoneimine from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase, thereby developing colour whose absorbance was taken at 546 nm.

Procedure

Cholesterol reagent 1(enzyme reagent) was constituted with 30 ml of distilled water. Ten microlitres (10µl) each of distilled water, standard, and serum was added into the test tubes marked blank, standard and test respectively. This was followed by the addition of 1 ml of cholesterol reagent to all the test tubes and incubated at room temperature for 10 min. The absorbance was read at 500 nm.

Calculation:

$$\text{Total cholesterol} = \frac{\text{Abs Test-Abs Blank}}{\text{Abs STD- Abs Blank}} \times \text{Concentration of STD (200 mg/dl)}$$

Where STD= Standard

Abs= Absorbance

2.2.7.2. Determination of serum high-density lipoprotein cholesterol (HDL)

Principle

The determination of serum HDL was based on the principle that very-low-density lipoproteins (VLDL) are precipitated in serum with phosphotungstate and magnesium ions. The HDL-CHOL is then measured spectrophotometrically by means of coupled reactions with 4- amino antipyrine in the presence of cholesterol esterase, oxidase, and peroxidase to form quinone imine.

Procedure

Two hundred microlitres (200 µl) each of sample and standard was added to the test tubes marked “Test” and “Standard respectively. This was followed by the addition of 500 µl (0.5 ml) precipitant (phosphotungstate and magnesium ions-reagent 2) to both test tubes. These were incubated at room temperature for 10 min. After this, the assay mixtures were centrifuged for 10 min at 400 rpm at room temperature using Ultra-Modern Centrifuge Machine (Model 800D, China). Fifty microlitres each of sample and standard supernatant were added into other sets of test tubes for test and standard while 50 µl (0.05 ml) distilled water was added into a test tube for blank. This was followed by the addition of 1 ml of cholesterol reagent to all the test tubes. These were mixed and incubated for 10 min at room temperature. The absorbance of the test and standard were measured against the reagent blank at 500 nm using spectrophotometer at room temperature.

Calculation:

$$\text{Concentration of HDL} = \frac{\text{Abs Test-Abs Blank}}{\text{Abs STD-Blank}} \times \text{Concentration of STD (200 mg/dl)}$$

2.2.7.3. Determination of ~~s~~Serum ~~t~~Triglycerides (TRIGS)

Principle

Triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

Procedure

One vial of triglycerides reagent 1b (enzyme reagent) was constituted with Fifteen mills (15 ml) of triglycerides reagent 1a (buffer reagent) at room temperature and labeled triglycerides reagent. Ten microlitres (10 µl) each of serum and triglycerides standard solution was added into the test tubes marked “Test” and “Standard respectively. This was followed

by the addition of 1 ml of triglycerides reagent to each of the test tubes including blank test tube and incubated at room temperature for 10 min. The absorbance was read at 500 nm.
Calculation:

$$\text{Concentration of triglycerides} = \frac{\text{Abs Test}}{\text{Abs STD}} \times \text{Concentration of STD (200mg/dl)}$$

2.2.7.4. Determination of low-density lipoprotein cholesterol (LDL)
Low-density lipoprotein was calculated from total cholesterol, triglycerides, and HDL cholesterol as described by Martin, [19].
Concentration of LDL= (TCHOL)-(HDL)-[TRIGS]/5(mg/dl)

2.2.7.5. Statistical analysis of results

Results obtained were expressed as mean ± SD of triplicate determinations. Results were analyzed using SPSS statistical software (version 25). Test of hypotheses was done by testing for the significant difference at a $P < 0.05$ level of significance.

3. RESULTS AND DISCUSSION

3.1. Results

Lethal acute toxicity (LD_{50}) of *Sida corymbosa* ethanolic-leaf extract carried was confirmed to be above 5,000 mg/kgbw.

3.1.1. Results of Serum Total Cholesterol Assay

The results of serum total cholesterol assay carried out on rats given *Sida corymbosa* ethanolic leaf extract before and after injecting CCL_4 are hereby presented in figures 2-3. Serum total cholesterol (TCHOL) levels were higher significantly ($P< 0.05$) in groups B1-B4 when compared with the treatment groups (Groups C1-E4, F1-H4) and groups A1-A4 (Negative control groups, normal rats),

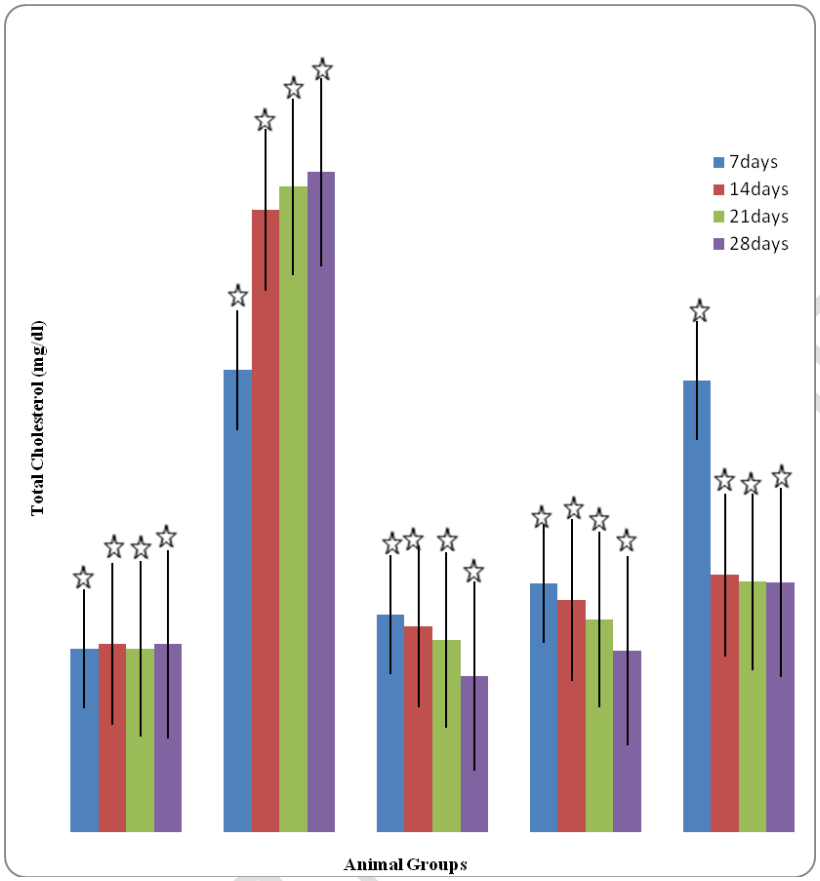

Figure 2: Graph of mean serum total cholesterol levels of rats given *Sida corymbosa* ethanolic-leaf extract before injecting CCl_4. Values represent means of triplicate results +/- SD. Test of significance was done at $P< 0.05$ level of significance.

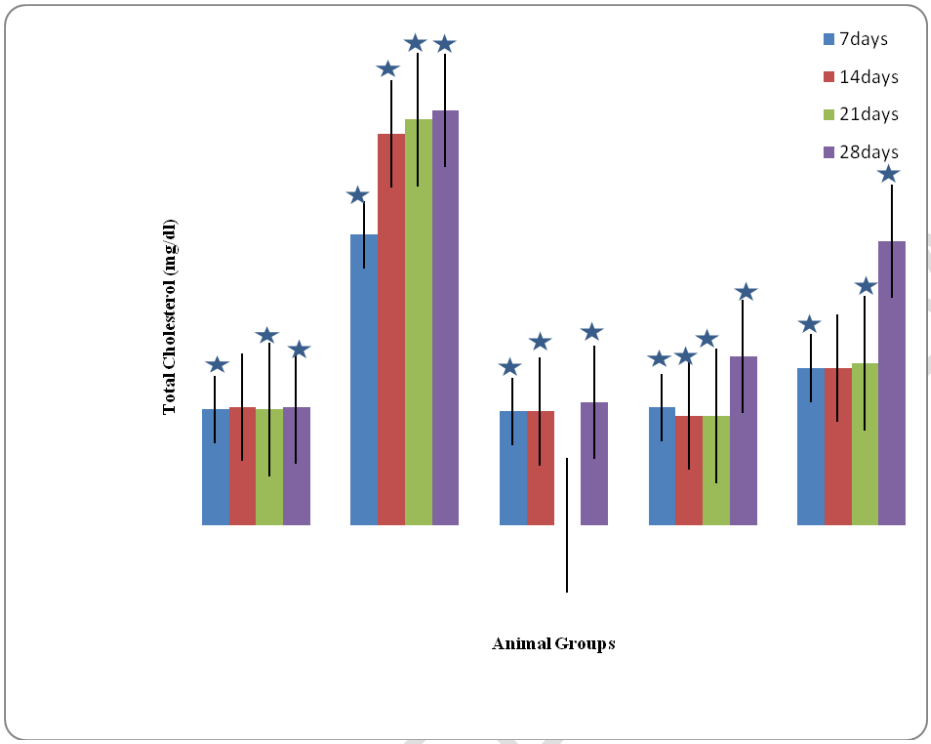

Figure 3. Graph of mean serum total cholesterol levels of rats given *Sida corymbosa* ethanolic-leaf extract after injecting CCl_4. Values represent means of triplicate results +/- SD. Test of significance was done at P< 0.05 level of significance.

3.1.2. **Results of Serum High Density Lipoprotein Assay of Rats Administered *Sida corymbosa* Ethanolic-Leaf Extract Before and After Injecting CCl4**

The results of serum high density lipoprotein cholesterol of rats administered *Sida corymbosa* extract before and after injecting CCl4 are hereby presented in figures 4-5.

Serum high density increased significantly among the treatments ($P < 0.05$) from 7-28 d of treatment when compared with the untreated groups-positive control (B1-B4) and normal rats-negative control (A1-A4) at 500, 250 and 100 mg/kgBW. Greatest increase was observed in rats given 500 mg/kgBW of the extract, followed with those given 250 mm/kgBW for both treatment groups (rats administered *Sida corymbosa* extract before and after injecting CCl4).

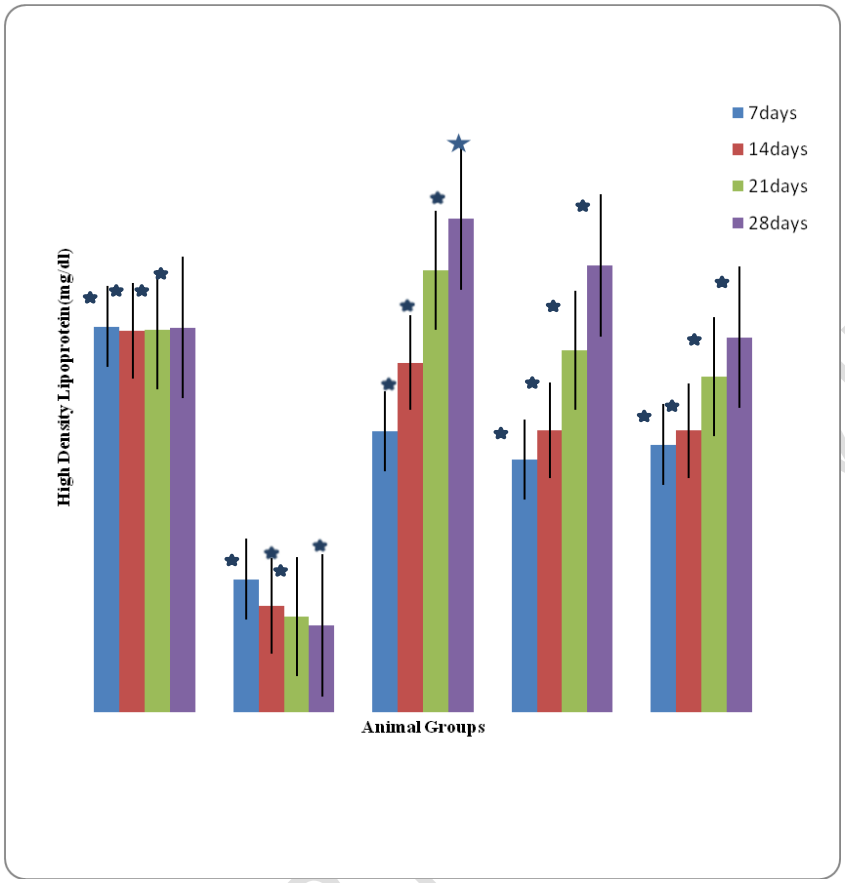

Figure 4: Graph of mean serum high density lipoprotein levels of rats given *Sida corymbosa* ethanolic-leaf extract before injecting CCl_4. Values represent means of triplicate results +/- SD. Test of significance was done at $P< 0.05$ level of significance.

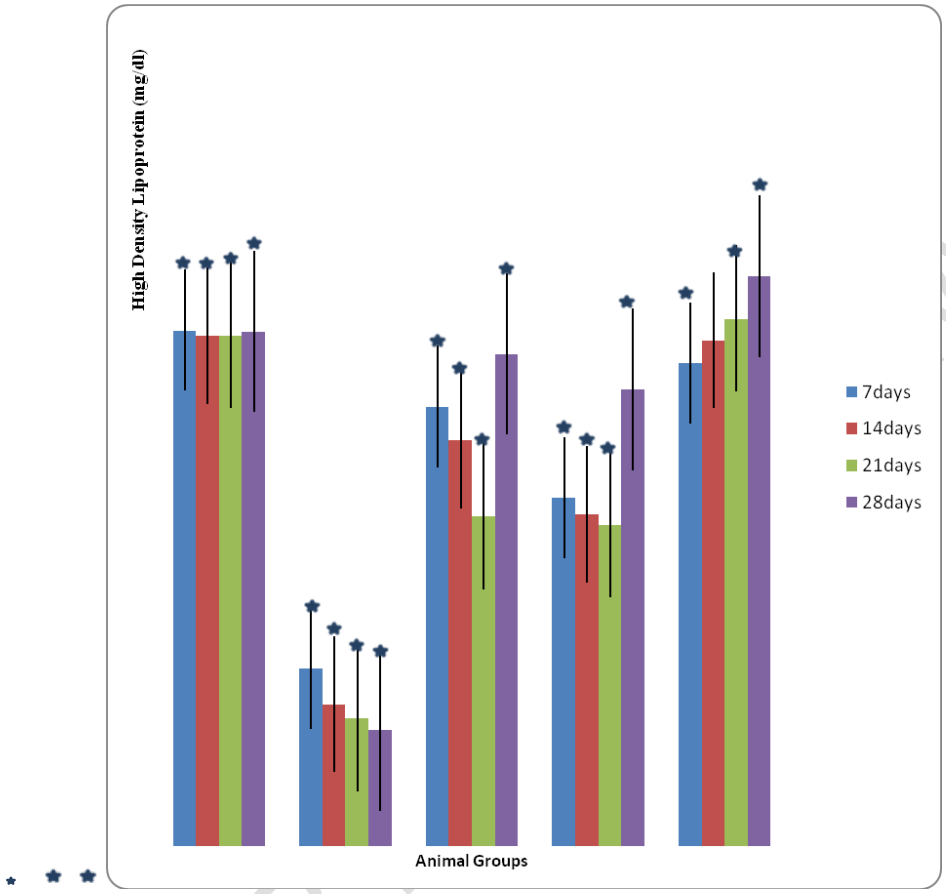

Figure 5: Graph of mean serum high density lipoprotein levels of rats given *Sida corymbosa* ethanolic-leaf extract before injecting CCl_4. Values represent means of triplicate results +/- SD. Test of significance was done at $P< 0.05$ level of significance.

3.1.3. **Results of Serum Low Density Lipoprotein Assay of Rats Administered *Sida corymbosa* Ethanolic-Leaf Extract Before and After Injecting CCl4**

The results of serum low density lipoprotein cholesterol of rats administered Sida corymbosa ethanolic-Leaf extract before and after injecting CCl4 are hereby presented in figures 6-7. It was observed that in rats administered administered *Sida corymbosa* ethanolic-Leaf extract before and after injecting CCl4, serum LDL levels decreased significantly ($P < 0.05$) in both treatment groups at 500, 250 and 100 mg/kgBW when compared with the positive control (rats injected CCl4 without

any treatment-B1-B4) and negative control (normal rats-rats given only feed and water) from 7-28 d of treatment. The greatest decrease among the treatment groups was observed on rats given 500 mg/kgBW of *Sida corymbosa* extract, followed by those given 250 mg/kgBW while those given 100 mg/kgBW of the extract was the least.

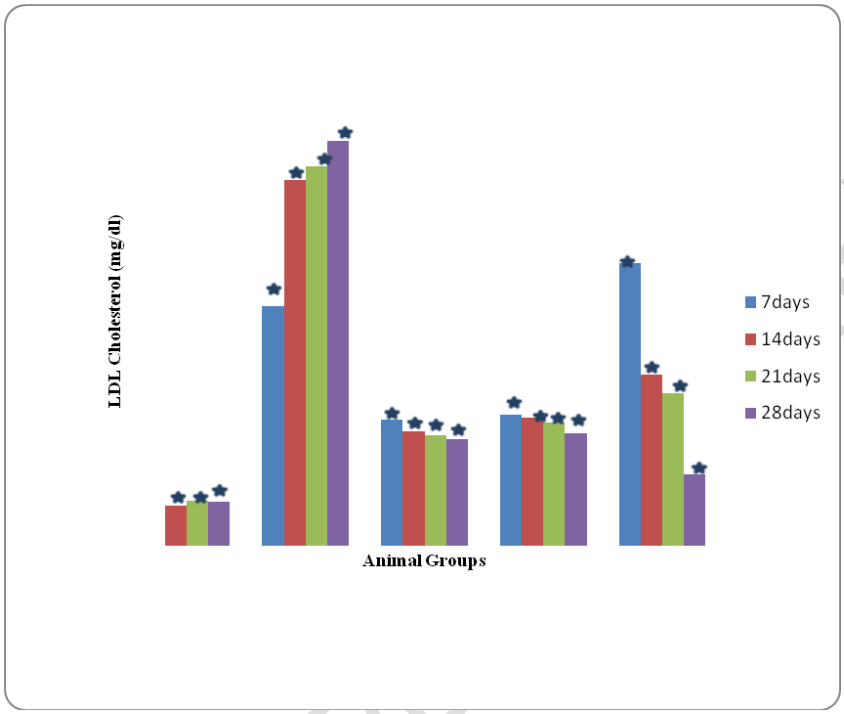

Figure 6: Graph of mean serum low-density lipoprotein-cholesterol levels of rats given *Sida corymbosa* ethanolic-leaf extract before injecting CCl_4. Values represent means of triplicate results ± SD. Test of significance was done at 0.05 level of significance.

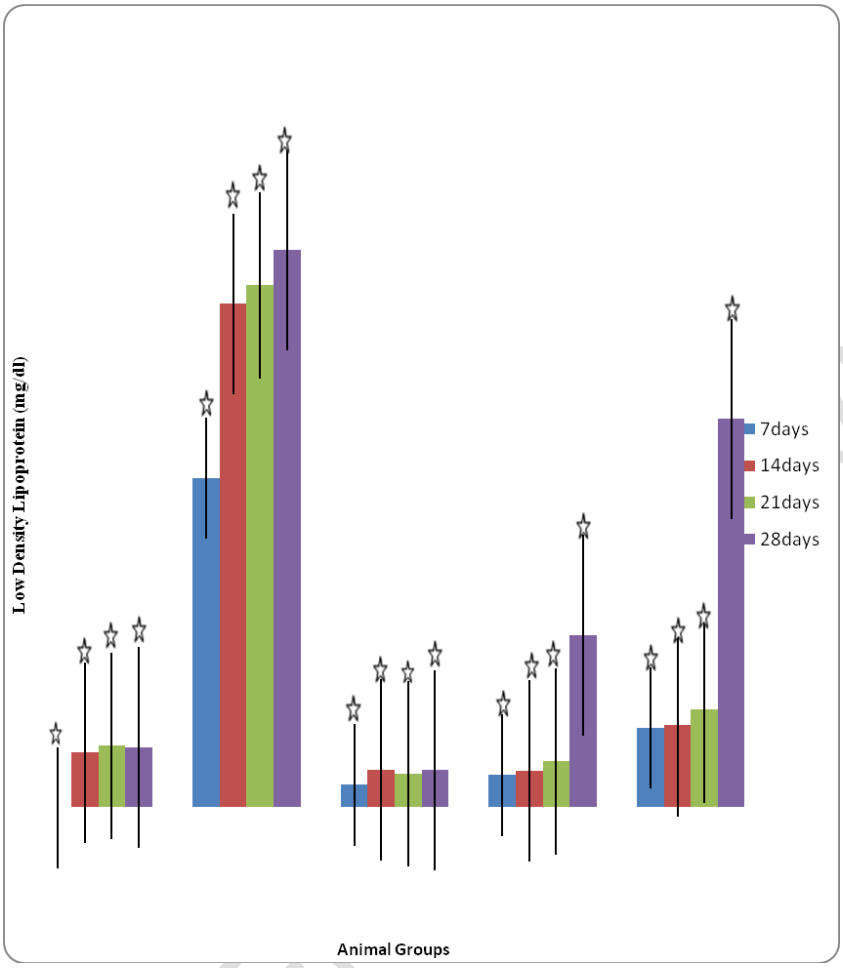

Figure 7: Graph of mean serum low-density lipoprotein-cholesterol levels of rats given *Sida corymbosa* ethanolic-leaf extract after injecting CCl_4. Values represent means of triplicate results ± SD. Test of significance was done at 0.05 level of significance.

3.1.4. **Results of Serum Triglycerides Assay of Rats Administered *Sida corymbosa* Ethanolic-Leaf Extract Before and After Injecting CCl4**

The results of serum triglycerides of rats administered *Sida corymbosa* ethanolic-Leaf extract before and after injecting CCl4 are hereby presented in figures 8-9. It was observed that in rats administered Sida *corymbosa* ethanolic-Leaf extract before and after injecting CCl4, serum triglycerides levels decreased significantly ($P < 0.05$) in both treatment groups at

500, 250 and 100 mg/kgBW when compared with the positive control (rats injected CCl4 without any treatment-B1-B4) and negative control (normal rats-rats given only feed and water) from 7-28 d of treatment. The greatest decrease among the treatment groups was also observed in rats given 500 mg/kgBW of *Sida corymbosa* extract, followed by those given 250 mg/kgBW while those given 100 mg/kgBW of the extract was the least.

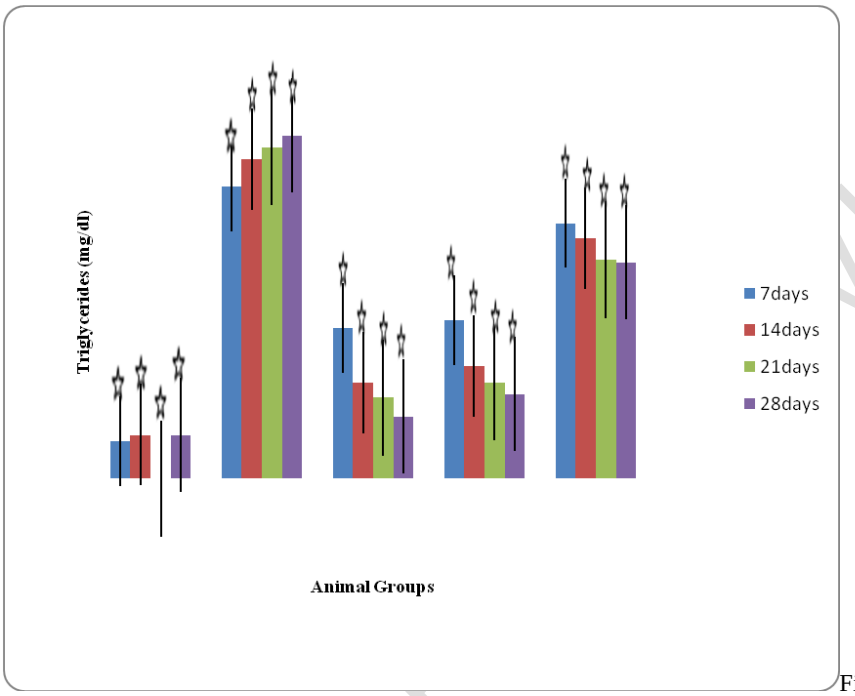

Fig. 8. Graph of mean serum triglycerides levels of rats given *Sida corymbosa* ethanolic-leaf extract before injecting CCl_4. Values represent means of triplicate results ± SD. Test of significance was done at 0.05 level of significance.

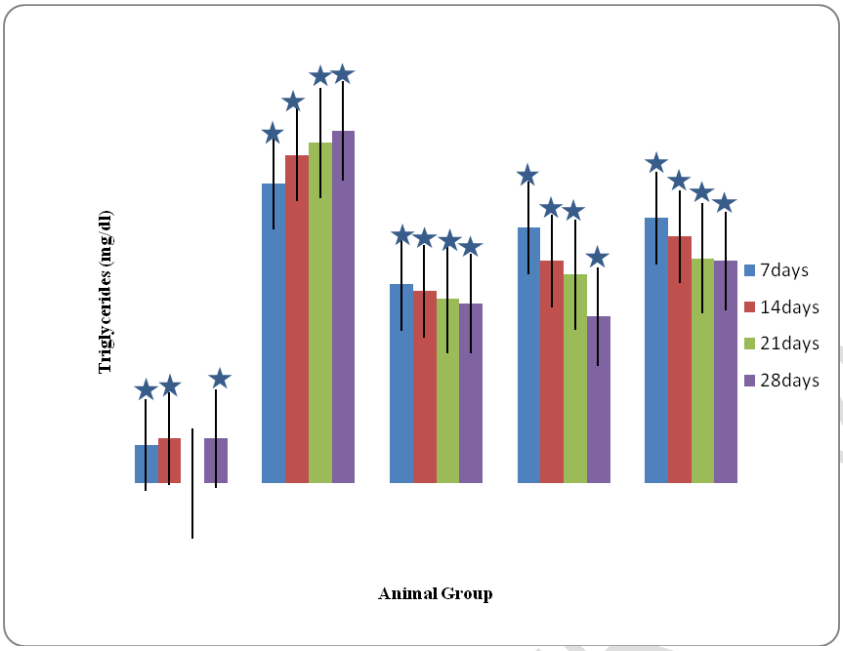

Fig. 9. Graph of mean serum triglycerides levels of rats given *Sida corymbosa* ethanolic-leaf extract before injecting CCl_4. Values represent means of triplicate results ± SD. Test of significance was done at 0.05 level of significance.

3.2. **Discussion**

This agrees with the reports of [11] on "Protective and curative potentials of *Sida corymbosa* extract against antioxidant depletion in rats administered carbon tetrachloride" and Hypoglycemic and hepatocurative activities of Aju-Mbaise on alloxin-diabetic model in male Wistar rat". Oral toxicity tests (LD_{50}) have been used as a parameter for determining the toxic level of a drug [20]. This suggests that the extract may not be toxic up to 5,000 mg/kgbw. The LD_{50} falls within the dose range for non-toxic chemicals as recommended by the Organization for Economic Cooperation and Development [21]. This means that the plant extract may have very low or no toxicity.

The decrease in serum total cholesterol on both treatment groups (figures 2-3) may be as a result of the treatment with extract as the phytochemical content of the plant's extract such as flavonoid may have reduced the amount of total cholesterol in the blood. This assertion is collaborated with the findings of [3, 8] on total cholesterol of blood. Dike [9] had reported in his work on phytochemical analysis that *Sida corymbosa* ethanolic and aqueous extracts contained flavonoids which had been reported to lower blood cholesterol [22]. This suggests that the treatments with the extract for both curative and protective effects are effective. Cholesterol synthesis in the treatment groups may have been inhibited by the presence of flavonoid, leading to decreased levels in the serum. Total cholesterol is one of the major parameters being determined when checking for abnormal lipid profiles and it is a risk factor to arthreosclosis and other age related diseases.

However, high density cholesterol levels have been shown by the results of this work so far to have elevated among those rats injected CCl4 before and after administering *Sida corymbosa* ethanolic-leaf extract, and decreased in groups injected CCl4 only (see figures 4-5), suggesting recovery of the affected rats from hyperlipidemic effects induced by CCl4. Increased HDL as witnessed in this work may have facilitated the transport of cholesterol to the liver from where it is excreted from the cells. This agrees with the similar work of [3]. This may be due to the phytochemical contents of the plant's extract. High density lipoprotein have been known to be good cholesterol [8]. Dike [9] had report that *Sida*

corymbosa ethanolic-leaf extract contains a lot of antioxidants such as flavonoids, anthocyanin, kaempferol, catechin, epicatechin and rutin. These antioxidants may have helped to raise HDL level in the blood and thereby lower LDL and total cholesterol levels [3]. Elevated HDL may have countered the effects of oxidized LDL.
Furthermore, the LDL assay carried out so far revealed that *Sida corymbosa* ethanolic-leaf extract at 500, 250 and 100 mg/kgBW reduced the levels of LDL in rats injected CCL before and after giving the extract from 7-28 d of treatment while the levels of LDL in rats injected CCl4 without giving any extract increased from 7-28 d of treatment (see figures 6-7). This is suggesting that the increased HDL level in rats given *Sida corymbosa* ethanolic-leaf extract may have lowered the levels of LDL in rats injected CCL before and after extract administration. This assertion is collaborated by the findings of [3, 8,20]. High density cholesterol helps to remove LDL cholesterol from the blood and transfer it to the liver from where it is excreted [21,22]. Low density cholesterol is known not to be a good cholesterol. Elevated level of LDL in the blood is a risk factor for heart disease and related illness [22,23]. The abnormal LDL levels in the blood induced by CCl4 may have reversed. The continuous increase in LDL levels in rats injected CCl4 only from 7-28 d may be an indication that the abnormal lipid profile induced in these groups is not being reversed.

Findings of this work also revealed that the levels of triglycerides in rats injected CCl4 before and after giving *Sida corymbosa* ethanolic-leaf extract decreased suggesting reversal of abnormal lipid profile induced by CCl4. Again, the antioxidants contained in this plant's extract may have contributed to this. This is suggesting recovery from the treated animal with Sida corymbosa. Triglycerides circulate in the blood and elevated triglycerides is also a risk factor to heart diseases and pancreatitis.

3.3. CONCLUSION

Sida corymbosa ethanolic-leaf extract may therefore have the potential to treat and prevent abnormal lipid profiles (hyperlipidemic effects) in rats injected with carbon tetrachloride since the levels of TCHOL, LDL-CHOL, and TRIGS decreased while HDL levels increased when given the extract. The extract may also be used to prevent hyperlipidemia by CCl_4 in rats. The treatment may equally be dose and duration-dependent, with 500 mg/kgbw being more effective at 28 d than 250 and 100 mg/kgBW. The findings of this study may not yet be extrapolated to humans until epidemiological studies are conducted on humans.

ETHICAL APPROVAL

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Ethical Committee of Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University Nnewi, Anambra State and the Ethical Approval number given as **APP/NAU/75**

All manuscripts which deal with the study of human subjects must be accompanied by Institutional Review Board (IRB) or Ethical Committee Approval, or the national or regional equivalent. The name of the Board or Committee giving approval and the study number assigned must accompany the submission. If required, author should be ready to submit a scanned copy of the IRB or Ethical Committee Approval at any stage of publication (Pre of post publication stage).

DEFINITIONS, ACRONYMS, ABBREVIATIONS

1. **CONC = CONCENTRATION**
2. **ABS = ABSORBANCE**
3. **STD = STANDARD**
4. **SD = STANDARD DEVIATION**

TERM: DEFINITION FOR THE TERM

REFERENCES

1. Geyue QU~~, Zhang,~~ Zhang C, Hong Z. Discordance between apolipoprotein B or non-HDL-cholesterol and LDL-cholesterol in middle-aged and elderly Chinese patients predicts arterial stiffness. *Lipids in Health and Dis*. 2021; 20:80.https://doi.org/10.1186/s12944-02-01509-6

2. Saibin W. Association between serum low-density lipoprotein cholesterol and metabolic syndrome in a working population. ~~*Lipids Health*~~*Lipids Health and Dis*. 2021; 20:73.https://doi.org/10.1186/s12944-021-01500-1

3. Sahar S, Nafiseh K, Zahra S~~, Javad~~, Javad N. The acute effect of incorporating lettuce or watercress into a moderately high-fat meal on postprandial lipid, glycemic response and plasma inflammatory cytokines in healthy young men: a randomized crossover trial. *Lipids Health Dis*. 2021; 15 (1): 66

4.Qingan F, Lijuan H, Yuan X, Yingping Y. Long Jiang. High lipoprotein(a) concentrations are associated with lower type 2 diabetes risk in the Chinese Han population: a large retrospective cohort study. Lipids in Health and Dis. 2021; 20:76. https://doi.org/10.1186/s12944-021-01504-x

5. Erik F. The effects of fat consumption on low-density lipoprotein particle size in healthy individuals: a narrative review. Lipids in Health and Dis. 2021; 20:56

6. Nariman M, Fatima Z F, Akram V, Abbas B A, Soraya S, Reza F. Serum levels of asprosin. Lipids in Health and Dis. 2021; 20:80. https://doi.org/10.1186/s12944-02-01509-6

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

7. Amanda J B, Alexandra M O, Salimah Z S, Robert A H and Kristin K C. Incidence, predictors and pattern of care of patients with very severe hypertriglyceridemia in Ontario, Canada: a population-based cohort study. Lipids in Health and Dis. 2021; 20:98.https://doi.org/10.1186/s12944-021-01517-96

8. Okonkwo COJ, Maduka HCC, Dike CC, Oguaka VN, Maduka SO and Ikeotuonye C M. The Effect of *Dacroydes edulis* (African Pear) Pulp Oil Extract on Serum Lipid Parameters in Male Albino Wistar Rats. *Journal of Applied Life Sciences International*. 2018; 17 (1): 1-8.

9. Dike CC, Ezeonu FC, Maduka H CC, Ezeokafor EN, Oguazu CE. Hepatoprotective potentials of *Sida corymbosa* (broom weed) ethanolic leaf extract against carbon tetrachloride (CCl_4)-induced hepatotoxicity on male Albino rats. J Pharmacogn Phytochem. 2018a; 7 (6): 2166-75.

10. Dike CC, Ezeonu FC, Maduka HCC. The effects of *Sida corymbosa* leaf extract against abnormal bilirubin and total protein in Albino rats by carbon tetrachloride (CCl4). *Journal of Diseases and Medicinal Plants*. 2020; 5(2): 39-44.

11. Dike CC, Ezeonu FC, Oguazu CE, Ezeokafor EN, Maduka HCC. Protective and curative potentials of *Sida corymbosa* extract against antioxidant depletion in rats administered carbon tetracjhloride. *American Journal of Biomedical and Life Sciences*. 2021; 9(2):97-110.

12. Dike CC, Ezeonu FC, Maduka H CC, Ezeokafor EN, Oguazu CE. Hepatotherapeutic potentials of *Sida corymbosa* (Wire Weed) ethanolic extract on carbon tetrachloride (CCl_4)-induced acute hepatotoxicity on male Albino wistar rats. *Europian J Pharm Med Res*. 2018b; 5 (12): 146-155.

13. Dike CC, Maduka HCC, Ezeokafor EN, Ezeonu FC. Phytochemical and Elemental Analysis of *Sida corymbosa* (Broom Weed or Wire Weed) Leaf and Ro_ot Extracts. Chem Sci Int J. 2018c; 24 (4): 1-9.

14. Abart JK, Kumar S, Mohanty A. Ethnomedicinal, phytochemical and ethnopharmacological aspects of four medicinal plants of Mmalvaceae used in Indian traditional medicine: A review. Medicines (Basel). 2017; 4(4): 75.

Dike CC, Ezeonu FC, Maduka HCC. The effects of *Sida corymbosa* leaf extract against abnormal bilirubin and total protein in Albino rats by carbon tetrachloride (CCl4). *Journal of Diseases and Medicinal Plants*. 2020; 5(2): 39-44.

15. Sundarganapath R, Niraimathi V, Ananda T, Jambulingm M, Narasimhan B, Aakash D. Phytochemical studies and pharmacological screening of *Sida rhombifalia* Linn. *Hygeia Journal of Dental Medicine*. 2013; 5(1): 19-22

16. Enegide C, David A, Fidelis SA. A new method for determining acute toxicity in animal models. Toxicol Int. 2013; 20(3):224-226.

17. World Health Organization. Recommendations for the preparation, characterization and establishment of international and other biological reference standards. WHO Experimental Committee on Biological standardization. 2004; Fifty-fifth report. WHO Technical Report Series No 932. http://www.whoint/bloodproducts/norms/en/

18. Parasaraman R, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacothera*. 2017; 8(3):153.

19. Martin AC. Clinical Chemistry and Metabolic Medicine. In: Hodler A and Edward A. (eds). Lipoprotein. 7th ed; Bookpower; 2006.

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

20. Ifedi IC, Ezeokafor, EN, Dim, CN, Dike CC, Eyeghre, OA, Hudu AA~~, Okoye~~, Okoye, KP. Hypoglycemic and hepatocurative activities of Aju-Mbaise on alloxine-diabetic model in male Wistar rat. *International Journal of Science Academic Research*. 2021; 2(5): 1515-1520.

21. Organization for Economic Cooperation and Development. Guidance document on mammalian reproductive toxicity testing and assessment, series on testing and assessment. 2008; No. 43. ENV/JM/MONO 16.

22. Xia F, Hongya W, Ting Y, Xiaozhen T, Yan Z, Jiahao F, Yong X. Low serum maresin-1 levels are associated with non-alcoholic fatty liover diseases: a cross-sectional study. Lipids in Health and Dis. 2021; 20:96.

23. Ruo-Lan L, Ling-Yu W, Shuqin L, Hu-Xinyue D, Qing Z, Ting Z, Wei P, Yongliang H, Chunjie W. Natural flavonoids derived from fruits are potential agents against atherosclerosis. Front Nutr. 2022; 9. https://doi.org/10.3389/fnut.2022.862277

Formatted: Font: Italic