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Effects Of Aqueous And Methanolic Stem Bark Extracts Of *Cissus Populnea* On Poloxamer 407 Induced Hyperlipidemia In Albino Rats

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Abstract: ***Plants have served human beings as a natural source for treatments and therapies from ancient times, among them medicinal herbs have gain attention because of its wide use and less side effects. This study was carried out to determine the effect of aqueous and methanolic stem bark extract of Cissus populnea on Poloxamer407 induced Hyperlipidemia in Albino rats. The plant material was collected and extracted using solvents (water and methanol) after which phytochemical analysis was screened. Thirty five (35) male and female albino rats weighing between 150-200 grams were divided into 7 groups. Group 1 normal control, 2-7 induced with poloxamer 407. Group 2(not treated), group 3 treated with standard drug (atorvastatin), group 4 and 5 received graded dose (100 and 200mg/kg/d) aqueous extract of Cissus populnea, group 6-7 received graded dose (100 and 200mg/kg/d) methanolic extract of Cissus populnea respectively for 14 days. Rats of all groups were sacrificed on the 15th day after treatments, blood was collected, allowed to clot for centrifugation separation into cells and serum. Separated Sera were used for Lipid profiles, liver function and antioxidants estimation. The data obtained were statistically analyzed using one way analysis of variance and t-test. Results showed that, poloxamer 407 significantly increased serum levels of Total Cholesterol, Triglycerides, Low Density Lipoprotein, Aspartate Transaminase, Alanine Transaminase, Alkaline Phosphatase and Milondialdehyde and decreased High Density Lipoprotein, Superoxidedismutase and Catalase. Aqueous and methanolic stem bark extracts of Cissus populnea significantly (P≥0.05) decreased the serum levels of, Low Density Lipoprotein - Cholesterol and triglycerides when compared with the positive control group as well as significantly (P≤0.05) increased High Density Lipoprotein level. The administration of aqueous and methanolic stem bark extracts of Cissus populnea significantly (P≥0.05) decreased the Alanine Transaminase and Aspartate Transaminase activity when compared with positive control group. Malondialdehyde is a biomarker of reative oxygen species where as catalase an antioxidant enzyme that mop up free radicals. The significant increase in serum level of superoxide dismutase and catalase reduce oxidative damage caused by oxidative stress resulting from free radicals generated in the body. The presence of bioactive compounds in the plant reduced cholesterol level and stimulates antioxidants in mopping up free radicals.***

Keywords: ***Cissus populnea, Poloxamer 407, Hyperlipidemia, Lipid profiles, Liver enzymes, Antioxidants.***

I. INTRODUCTION

Cardiovascular disease is leading cause of death in the world (Pankaj *et al.,* 2010). Hyperlipidemia is a collective term used to describe human conditions when a plasma level of one or more classes of lipids, namely cholesterol, triacylglycerides, phospholipids and fatty acids increases above normal levels. Hyperlipidemia is one of the major causes of the development of cardiovascular disorders such as atherosclerosis, myocardiac infarction. It account for about

56% of stroke, 18% of ischemic heart disease and more than 4 million death per year globally (WHO, 2002). In Nigeria, it accounts for about 45% to 73% death per year (Raida *et al*., 2008; Asheneni, 2014).*Cissus populnea* belong to the family of Vitaceae and the genus *Cissus* which comprises of about 350 species, it is native to west tropical Africa. In Nigeria it is commonly found in the Northern and Southern parts. The vernacular names include food gum (English), 'Ogbolo ajara'(Yoruba) and 'Dafaaraa'(Hausa) 'Okoho'(Idoma and Igala), (Burkill, 2000). The plant is a strong woody climbing shrub, 8-10cm long and with 7½ cm in diameter, with a perennial root stock with jointed stems often with watery juice. Bioactive ingredient made from the plant has proven medicinal properties. Experts in the evaluations of *Cissus populnea*, reported that they have antimicrobial activities which cure many sexually transmitted diseases infections that could be responsible for male infertility (Ojekale *et al.*, 2006). Other studies have also shown that the essential oil from the stem powder of the plant inhibit the growth of several germs of bacteria origin and as such may correct male infertility arising from the bacterial infections. According to Belmain *et al.,(* 2000) the plant is used as diuretic and as a post- harvest ethnobotanical protectant in Ghana. Previous studies on the plant have also shown that the root extracts of the plant have been used for the treatment of skin diseases, boils, infected wounds and for treating urinary tract infection, thus suggesting anti bacteria potency of the plant (Kone *et al.*, 2004). The root is also used in the southern part of Nigeria as an arrow – poison antidote (Gill, 1992). In Niger, Kogi, Plateau, Kwara and Benue state of Nigeria, the plant is used for making vegetable soup for post natal stoppage of blood flow (Soladoye and Chukwuma, 2012).

Local residents with limited access to medical technology and equipment benefit from traditional remedies, which can form an effective indigenous healthcare system. Research about medicinal plants is significant in revealing important traditional medicinal plant species, often leading to the discovery of new drugs, and contributing to the local economy. Millions of people in the developing world rely on traditional medicinal plants for primary healthcare, skin care, economic benefits, and cultural development (Cordell and colvard, 2012). In areas where medical facilities are underdeveloped, traditional medicinal plants are especially important.

II. MATERIALS AND METHODS

MATERIALS

EQUIPMENTS

Conical flask, test tubes, hand gloves, EDTA tubes, spatula, micropipette, Beakers, Electronic weighing balance, Colorimeter, mortar and Pestle, Water bath, refrigerator, centrifuge, stirrer, Buchner funnel, scissors, wooden cage, whatman No. 1 filter paper, syringes and needles, glass bottle, razor blades, digital spectrophotometer, cuvette, rotator and sieve.

REAGENTS

Methanol, Distilled water, Chloroform, Buffer, Ethanol, N-hexane, Silica gel, sodium hydroxide, glacial acetic acid, ethyl acetate ascorbic, hydrochloric acid, ferric chloride, olive oil, acetic anhydrides, sodium tetraoxosulphate (vi) acid, dilute ammonia solution, chloroform, mayer's reagent and poloxamer 407.

COLLECTION OF PLANT MATERIALS

The *Cissus populnea* stems was obtained from the forest at Aila in Agatu Local Government Area of Benue State. It was the thoroughly washed to remove the adhering dirts and foreign material. The fresh stems was cut into five strips with a kitchen knife and will be air dried at room temperature. It was then reduced to a fine powder using mortar and sieve. The powdered sample was put inside an air-tight polyethyhene container, neatly labeled and kept in a refrigerator pending analysis.

METHODS

PREPARATION OF PLANT EXTRACT

The aqueous and methanolic stem bark extract was prepared according to the method by Salahdeen and Yemitan(2006). The powder was weighed using an electronic weighing balance and was soaked in 80% solvents (distill water and methanol) at the ratio of 1: 8 of powder to solvents. The mixtures were kept at room temperature for 48 hours (2 days). The extracts were filtered using a cheese cloth and then a filter paper. The extracts were finally evaporated to dryness using a water bath. The percentage yield of the stem bark extracts were determined.

PERCENTAGE (%) YIELD OF EXTRACT

Percentage yield: After filtration and concentration, the concentrated aqueous extracts, gave a total yield of 39.82% (39.82 g) and 36.34% (36.34g) for aqueous and methanolic stem bark extract of *cissus populnea*.

Percentage yield of Aqueous stem bark extract = $\frac{39.82g}{200g} \times \frac{100}{1} = 19.915\%$

Percentage yield of methanolic stem bark extract = $\frac{36.35g}{200g} \times \frac{100}{1} = 18.17\%$

III. EXPERIMENTAL ANIMALS

A total of thirty five (35) albino rats (male and female) weighing 150g to 200g were used for this study. The animals were allowed two weeks acclimatization, after which they were reweighed and housed in wooden cages with wooden bottom and wire-mesh top, under normal controlled environmental conditions of temperature and 12 hours light/dark cycle. Water and food was provided throughout the experimental period.

ANIMAL GROUPING AND EXPERIMENTAL PROTOCOL

The phase consists of thirty five (35) animals, assigned into seven (7) groups of five animals each.

ANIMALS GROUPING AND TREATMENTS

GROUP 1: Normal control (not induced)
GROUP 2: Positive control.
GROUP 3: Standard control.
GROUP 4: Treatment with 100mg/kg body weight of Aqueous extract.
GROUP 5: Treatment with 200mg/kg body weight of Aqueous extract.
GROUP 6: Treatment with 100mg/kg body weight of methanolic extract.
GROUP 7: Treatment with 200mg/kg body weight of methanolic extract.

INDUCTION OF HYPERLIPIDEMIA

Hyperlipidemia was induced with the method described by Megallic *et al.*,(2005). 1000mg/kg dose of Poloxamer 407 was introduced intraperitoneally. To confirm the induction of hyperlipidemia, 24 hours after induction blood sample was collected from the rat tail in each group and assayed for; triacylglycerol (TAG) and total cholesterol (TC). TC and TAG levels above borderline of (200-239) mg/dl and (150-199) mg/dl respectively were considered hyperlipidemic (Hetal *et al*., 2013; Asheneni, 2014).

COLLECTION OF BLOOD SAMPLES FOR ANALYSIS

The administration of extracts lasted for the period of 15 days after which the animals were sacrificed twelve hours after the last administration in accordance with the guidelines of the European Convention for the protection of Vertebrate animals and other scientific purposes ETS-123 (European Treaty Series, 2005). Whole blood was collected from the heart via cardiac puncture using sterile syringe and needle. The blood samples were put into plain sample tubes. The blood samples collected were transfered into EDTA bottles and then corked immediately, shaken gently to allow the blood mix with the anti coagulant and prevent clotting and the blood samples were used to check the effects of *Cissus populnea* on lipid profiles, enzyme assay and antioxidants.

BIOCHEMICAL ASSAYS (LIPID PROFILE, LIVER ENZYMES AND ANTIOXIDANTS)

ASSESMENT OF LIPID PROFILE

Total Cholesterol (TC)

The serum level of total cholesterol was quantified by methods described by Stein (1987), using assay kits (Randox Laboratories Ltd). The value of TC present in the serum was expressed in mg/dl.

PRINCIPLE: A large portion of the cholesterol in blood is in the form of cholesteryl esters, which is hydrolysed by cholesterol esterase into cholesterol. Cholesterol is then oxidized by cholesterol oxidase to yield H2O2. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase which can be detected spectrophotometrically.

Cholesterol ester + H2O $\xrightarrow{\text{cholesterol Esterase}}$ Cholesterol + Fatty acids

Cholesterol ester + O2 $\xrightarrow{\text{cholesterol oxidase}}$ Cholestene-3-one + H2O2

2 H2O2 + Phenol + 4-Aminoantipyrine $\xrightarrow{\text{perxidase}}$ Quinoneimines + 4 H2O

PROCEDURE: Exactly, 1000µl of the cholesterol reagent which is made up of 4-Aminoantipyrine, phenol, peroxidase, cholesterol esterase, cholesterol oxidase and buffer was added into three clean test tubes labelled as test, standard and reagent blank containing 10µl serum, 10µl standard reagent and 10µl distilled water respectively. The content in each of the test tube was mixed and incubated for 5 minutes at 370C. The absorbance was read against the reagent blank at 500nm within 60 minutes.

Calculation; Using the standard, the concentration of cholesterol in the sample is given as:

Conc. Of cholesterol (mg/dl) = $\frac{A\,sample}{A\,standard} \times Conc.\,of\,standard$

ΔAsample = Change in absorbance of sample
ΔAstandard = Change in absorbance of standard.

Triacylglycerol (TAG)

The serum triacylglycerol level was determined by enzymatic method described by Stein (1987), using assay kits (Randox Laboratories Ltd). The values of TAG were expressed in the unit of mg/dl.

PRINCIPLE: The 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 (Tietz, 1990).

Triglycerides +H_2O $\xrightarrow{\text{Lipases}}$ Glycerol + Fatty acids

Glycerol + ATP $\xrightarrow{\text{Glycerol Kinase}}$ Glycerol -3-phosphate + ADP

Glycerol-3-phosphate +O2 $\xrightarrow{\text{Glycero Phosphate oxidase}}$ Dihydroxyacetone + Phosphate + H2O2

H2O2 +4-Aminophanazone +4-Chlorophenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + HCl + 4H2O

Reagent Composition:Rla. Buffer: Pipes Buffer (40mmol/l, pH7.6), 4-chloro-pheno (5.5mmol/l), Magnesium-ions (17.5mmol/l). Rlb. Enzyme Reagent: 4-aminophenazo

(0.5mmol/l), ATP (1.0mmol/l), Lipases (≥ 150U/ml), Glycerol-kinase (≥ 0.4U/ml), Peroxidase (≥ 0.5U/ml). CAL. Standard: (196mg/dl).

Reagent Preparation:One vial of Enzyme reagent R l b was reconstituted with 15 ml of Buffer Rl a.

PROCEDURE: Exactly, 1000µl of the tricylglycerols reagent which is made up of buffer and enzyme reagent was added into clean test tubes containing 10µl of serum, 10µl of standard, 1000µl of the tricylglycerols reagent was also added to empty test tube (serving as reagent blank). The content in each of the test tubes was mixed and incubated for 5 minutes at 37OC. The absorbance was read against the reagent blank at 500nm.

Calculation; Conc. of triacylglycerol (mg/dl) = $\frac{A\,sample}{A\,standard} \times Conc.\,of\,standard$

GPO = Glycerol-3-phosphate oxidase

POD = Peroxidase

ΔAsample = Change in absorbance of sample

ΔAstandard = Change in absorbance of standard

Serum High Density Lipoprotein-Cholesterol (HDL-c)

The serum levels of HDL-c were measured by the method of Wacnic and Albers (1978), using assay kits (Randox Laboratories Ltd). The values were expressed in the unit of mg/dl.

PRINCIPLE: Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined.

Reagent Composition: R1: Phosphotungstic Acid (0.55mmol/l), Magnesium Chloride (25mmol/l), and Concentration of standard (210mg/dl).

Reagent Preparation: The precipitating reagent was prediluted in the ratio 4 + 1 with redistilled water (the contents of 80 ml bottles was diluted with 20 ml redistilled water**).**

PROCEDURE: Exactly, 0.5ml of reagent1 (R1) which is made up of phosphotungstic acid and magnesium chloride was added into two clean test tubes; one containing 0.2ml of serum and the other containing 0.2ml of standard, the content in each test tubes was mixed thoroughly and allowed to stand for 10minutes at room temperature, centrifuged at 4000 rpm for 10 minutes and the supernatant was collected. 1.0ml of reagent 2 (cholesterol reagent) made up of phosphate, cholesterol esterase, cholesterol oxidase, peroxidase, 4-Aminoantipyrine, sodium cholate and dichlorophenolsulfonate was added into clean test tubes containing 0.05ml of sample supernatant, 0.05ml standard supernatant and an empty test tube (reagent blank), mixed thoroughly and incubated for 30 minutes at room temperature. The absorbance was read against the reagent blank at 500nm within 60 minutes.

Calculation; HDL-c Concentration (mg/dl) = $\frac{A\,sample}{A\,standard} \times Conc.\,of\,standard$

ΔAsample = Change in absorbance of sample

ΔAstandard = Change in absorbance of standard

Low Density Lipoprotein-Cholesterol (LDL-c)

The serum levels of (LDL-c) were calculated in mg/dl according to the protocol of Friedewald *et al*., (1972) using the equation below;

LDL-c = (TC - TAG/5) – HDL-c

LIVER FUNCTION ASSAY

Determination Of Aspartate Aminotransferase (AST) Activity

AST activity was determined by the method described by Amador and Wacker (1962).

PRINCIPLE: In this reaction L-Aspartate and α-Ketoglutarate react in the presence of AST in the sample to yield oxaloacetate and L-glutamate. The oxaloacetate is reduced by malate dehydrogenase to yield L-malate with the oxidation of NADH to NAD+. The reaction is monitored by measurement of the decrease in the absorbance of NADH at 340nm. The rate of reduction in absorbance is proportional to AST activity in the sample.

PROCEDURE: To 1 ml of reagent added to all required test tubes, 0.05 ml of the sample was added to the sample test tube and none to the blank. It was incubated at room temperature for 20 min, mixed immediately and first absorbance of test was read exactly at 1 minute and thereafter at 30, 60, 90 and 120 seconds at 340 nm. The mean change in absorbance per minute was determined and the test results were calculated. Serum AST activity (IU/L) = A /min × F.

A = Change in absorbance per minute

F = 3376 (Based on the millimolar extinction coefficient of NADH at 340 nm)

Determination Of Alanine Aminotransferase (ALT) Activity

ALT activity was determined by the method described by Amador and Wacker (1962).

PRINCIPLE: In this reaction, L-alanine and α-ketoglutarate react in the presence ALT in the sample to yieldPyruvate and L-glutamate. Pyruvate is reduced by lactate dehydrogenase to yield lactate with oxidation of NADH to NAD. The reaction is monitored by measurement of the decrease in absorbance at 340 nm. The rate of reduction is proportional to ALT activity in the sample.

PROCEDURE: To 1 ml of reagent added to all required test tubes, 0.05 ml of the sample was added to the test sample tube test and none to the blank. It was incubated at room temperature for 20 min, it was mixed immediately and first absorbance of test was read at exactly 1 minute and thereafter at 30, 60, 90 and 120 seconds at 340 nm. The mean change in absorbance per minute was determined and test results were calculated.

Serum ALT activity (IU/L) = A/min × F.

A = Change in absorbance per minute

F = 3376 (Based on the millimolar extinction coefficient of NADH at 340 nm)

Determination Of Alkaline Phosphatase (ALP) Activity

Serum activity of alkaline phosphatase (ALP) was determined by the method described by Haussament, (1977).

PRINCIPLE: P-nitrophenylphosphate + H_2O ALP phosphate + p-nitrophenol (405 nm)

Alkaline phosphatase in a sample hydrolyses paranitrophenyl phosphate into paranitrophenol and

phosphate, in the presence of magnesium ions. The rate of increase in absorbance of the reaction mixture at 405 nm and 37^0C due to liberation of paranitrophenol is proportional to the alkaline phosphatase activity.

PROCEDURE: Reagent (1ml) containing diethanolamine buffer, magnesium chloride and substrate (P-nitrophenylphosphate) was added into a clean test tube and incubated at 370C followed by the addition of 0.02 ml of sample. This was mixed thoroughly and immediately absorbance of test was read exactly at 30, 60, 90 and 120 seconds at 405nm against the reference blank (distilled water).The mean change in absorbance per minute was determined and the test results were calculated. Calculation: The ALP activity was calculated using the following formulae: Serum Alkaline phosphatase Activity (IU/L) = A/min x F.

A = Change in absorbance per minute

F = 2713 (calculated on the basis of molar extinction coefficient for paranitrophenol and ratio of total assay to sample volume).

Determination Of Some Antioxidants Parameters

Antioxidant activity of aqueous and methanolic stem bark extracts of *Cissus populnea* wasevaluated by procedure described by Galal *et al* (2012).

Estimation Of Malondialdehyde

The concentration of the concentration of malondialdehyde (MDA), a lipid peroxidation product (MDA) was determined by the method described by Slater and Sawyer, (1971).

PRINCIPLE: Malondialdehyde in plasma is one of the aldehyde products of lipid peroxidation which react with TBA to form a colored product, the absorbance of which is measured spectro-photometrically at 530 nm.

PROCEDURE: In the test tubes 0.5 ml of serum from test samples were taken, and 3 ml of 10% TCA was added to it, mixed well and the tubes were left to stand for 10 min at room temperature, and then centrifuged for 15 min at 5000 rpm. Two sets of test tubes were taken marked as blank and test. For a test sample, 2 ml of supernatant fluid was taken and added to 1.5 ml of 0.67% TBA. For a blank sample, 2 ml of distilled was added in 2 ml of 0.67% TBA. After mixing well and keeping in the boiling water bath for 10 min, they were cooled under tap water. A pale pink color developed, the color intensity was measured at 530 nm by colorimeter. Using the molar extension coefficient (1.5 × 105) and result was expressed as n moles of malonaldehyde (MDA)/100 ml of serum.

1.5 = 100 µmol/L (here, 100 is for conversion from ml to dl).

Then MDA = 100 × O.D. of unknown/1.5.

Estimation Of Superoxide Dismutase

The superoxide dismutase (SOD) activity was assayed by the method described by Marklund and Marklund, (1974).

PRINCIPLE: This method utilizes the inhibition of auto-oxidation of pyrogallol by superoxide dismutase (SOD) enzyme. The assay mixture in a 3 ml volume consisted of 100 µL each of 0.2 mM pyrogallol, 1 mM EDTA, 1 mM DTPA, and varying concentrations of standard SOD enzyme or 100 µL of serum in air equilibrated tris-HCl buffer (50 mM; pH 8.2).

PROCEDURE: The reaction mixture prepared in 3 sets includes standard, test and control. Pyrogallol was added after the addition of all other reagents to start the reaction. Initial 10 s period was considered as induction period of the enzyme. So after 10 s, change in absorbance at 420 nm at 10 s intervals was recorded to a period of 4 min. The average change in the absorbance per minute was calculated.

One unit of enzyme superoxide dismutase was defined as the amount of enzyme received to cause 50% inhibition of pyrogallol auto-oxidation. Accordingly, the activity of the enzyme in different standards was expressed in units/ml.

IV. STATISTICAL ANALYSIS

Data obtained was expressed as Mean Standard Deviation and was analyzed using the Analysis of Variance ANOVA; (Welkowitz, 1976) and students 't' test where applicable. Values at $p \leq 0.05$ regarded as significant in comparison with appropriate controls using Duncan multiple comparison.

V. RESULTS

EFFECTS OF AQUEOUS AND METHANOLIC STEM BARK EXTRACTS OF *CISSUS POPULNEA* ON LIPID PROFILES PARAMETERS

Oral administration of aqueous and methanolic stem bark extracts of *Cissus populnea* at 100 and 200mg/kg/d to both male and female rats for 14 days decreased the serum concentration of total cholesterol, triacylglyceride and LDL-c and increased serum level of high density Lipoprotein cholesterol. The decreased the serum level of total cholesterol was statistically not significant ($p \leq 0.05$) when compared with the positive control but statistically significant ($p \geq 0.05$) when compared with the normal control in group IV, V and VI of the graded doses whereas group VII was statistically not significant ($p \leq 0.05$) when compared with the both controls. On the contrary, 100 and 200mg/kg/d of aqueous and methanolic stem bark extracts of *Cissus populnea* induced not significant ($p \leq 0.05$) decrease in the triacyglycerol serum level. The increased serum HDL-cholesterol was significant ($p \geq 0.05$) in group IV and V when compared with the positive control and statistically not significant ($p \leq 0.05$) when compared with normal control of the treatment groups. It was further observed that treatment with the aqueous and methanolic stem bark extracts of *Cissus populnea* decrease in serum LDL-cholesterol. This was statistically not significant in the group treated with graded dose of 100 and 200mg/kg when compared with positive control and normal control as showed in Table 1.

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Category		N	SAT	
			Mean	Std. Error
Treatment	ID	114	49.01	1.296
	PDID	123	76.83	1.638
	TD	122	50.75	1.440
Mathematical	Low	92	61.31	1.370
Ability	Medium	226	58.89	.715
	High	41	56.40	1.980
Gender	Male	214	58.99	1.003
	Female	145	58.74	1.340

Values with superscripts down the column are significant difference.
Results are expressed in Mean ± Standard Deviation. One way ANOVA followed by student's "t" test.number of animals n=5.
Key: NC: Normal Control, PC: Positive Control, SC: Standard Control, AE: Aqueous Extracts ME: Methanolic Extracts, Trt: Treatment.
TC: Total Cholesterol, TG: Tryglycerides, HDL: High Density Lipoprotein, LDL: Low Density lipoprotein.
Table 1: Effects of aqueous and methanolic stem bark extracts of Cissus populnea on lipid profiles parameters

EFFECTS OF AQUEOUS AND METHANOLIC STEM BARK EXTRACTS OF *CISSUS POPULNEA* ON LIVER FUNCTION PARAMETERS

The result obtained in table 2 showed that the aqueous and methanolic stem bark extracts of *Cissus populnea* had effect on the serum ALT, AST and ALP levels of normal rats when compared with their controls.

The aqueous and methanolic stem bark extracts of *Cissus populnea* significantly increase the serum ALT, AST and ALP levels of treated rats when compared with their controls of the experiment as seen in table 2. The serum AST, ALT and ALP levels increased significantly (P≤0.05) in the positive control group when compared with their normal controls. The increase was most expressed on group II which was induced with poloxamer 407. While the most significant decrease was observed on the graded dose treatment groups of aqueous and methanolic stem bark extracts of *Cissus populnea* in serum ALP, AST and ALP levels in rats administered extracts as compared with the controls. The decreased in serum levels of ALP, AST and ALT in the treatment groups of aqueous and methanolic stem bark extracts of *Cissus populnea* were statistically not significant (P<0.05). These decrease were in group IV and VI of aqueous and methanolic stem bark extracts of *Cissus populnea* when compared with positive and normal control but statistically significant in group V and VII when compared with normal control for ALT. Also, in group V and VI of aqueous and methanolic stem bark extracts of *Cissus populnea* were significantly decrease (p≥0.05) when compared with positive. It is statistically not significant (P<0.05) when compared with normal control but statistically not significant (P<0.05) in group IV and VII when compared with positive control. Group IV and VII were significantly decrease (p≥0.05) when with normal control for AST. Whereas ALP serum levels in the treatment groups were statistically not significant (P<0.05) when compared with both positive and normal control.

Groups	Drug Dose Trt		ALT	AST	ALP
I	NC		122.09±10.51^{c}	183.35±23.91^{c}	59.61±15.43^{d}
II	PC		132.58±22.64^{d}	212.28±40.18^{e}	66.57±12.13^{e}
III	SC		106.40±8.58^{b}	107.88±49.28^{a}	48.60±36.99^{c}
IV	AE	100mg/kg	125.84±39.17^{c}	205.77±47.19^{b}	56.58±13.67^{d}
V	AE	200mg/kg	75.76±11.62^{a}	121.20±41.54^{b}	40.25±25.64^{c}
VI	ME	100mg/kg	124.92±21.00^{c}	204.31±3.54^{d}	38.23±22.31^{b}
VII	ME	200mg/kg	104.25±36.93^{b}	183.06±29.19^{c}	21.90±36.22^{a}

Values with superscripts down the column are significant difference.
Results are expressed in Mean ± Standard Deviation. One way ANOVA followed by student's "t" test.number of animals n=5.
Key: NC: Normal Control, PC: Positive Control, SC: Standard Control, AE: Aqueous Extracts ME: Methanolic Extracts, Trt: Treatment.
ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase.
Table 2: Effects of aqueous and methanolic stem bark extracts of Cissus populnea on liver function parameters

EFFECTS OF AQUEOUS AND METHANOLIC STEM BARK EXTRACTS OF *CISSUS POPULNEA* ON ANTIOXIDANTS PARAMETERS

Serum Malondialdehyde level increased in the positive group and was statistically not significant when compared with normal control. Serum Malondialdehyde levels were found to be reduced in the graded dose groups aqueous and methanolic stem bark extracts of *Cissus populnea* but they were also not statistically significant (P<0.05) in comparison with normal control group.

Decreased in serum Superoxide dismutase level was observed in positive control group and the difference was highly significant (P≥0.05) in decrease when compared with normal control group. Oral administration of graded doses of aqueous and methanolic stem bark extracts of *Cissus populnea* increased serum Superoxide dismutase levels when compared with positive control group. The highest increase was found in group VII of methanolic extract of *Cissus populnea* (200 mg/kg) group, while aqueous and methanolic stem bark extracts of *Cissus populnea* in group IV, V and VI showed very significant (P≤0.05) increase in comparison with positive control group and statistically not significant (P<0.05) when compared with normal control.

Serum Catalase level was found to be increased in normal control and all four extract groups in a graded manner in comparison with the positive control group. These increased were statistically not significant (P<0.05) in the group V, VI and VII and statistically significant in comparison with normal control groups IV.

Groups	Drug Dose Trt		MDA(nmole/ml)	SOD(U/ml)	CAT(U/ml)
I	NC		14.73±1.43^{c}	70.66±4.87^{c}	77.50±11.55^{c}
II	PC		17.49±1.76^{e}	53.03±03.48^{a}	51.03±16.73^{a}
III	SC		14.96±01.98^{c}	64.16±03.58^{b}	52.03±16.91^{a}
IV	AE	100mg/kg	15.46±3.05^{d}	58.19±3.61^{a}	63.78±21.54^{b}
V	AE	200mg/kg	13.03±.94^{b}	66.81±3.66^{b}	73.78±9.80^{c}
VI	ME	100mg/kg	14.50±0.78^{c}	51.64±2.59^{a}	50.01±19.99^{a}
VII	ME	200mg/kg	12.24±3.20^{a}	65.75±6.99^{b}	58.33±6.53^{a}

Values with superscripts down the column are significant difference.

Results are expressed in Mean ± Standard Deviation. One way ANOVA followed by student's "t" test.number of animals n=5
Key: NC: Normal Control, PC: Positive Control, SC: Standard Control, AE: Aqueous Extracts ME: Methanolic Extracts, Trt: Treatment.
MDA: Malondialdehyde, SOD: Superoxidedismutase, CAT: Catalase.

Table 3: Effects of aqueous and methanolic stem bark extracts of Cissus populnea on antioxidants parameters.

VI. DISCUSSION

Earlier reports states that certain phytocomponents, particularly saponins, exhibit antihyperlipidaemic and hypocholesterolaemic actions by inhibiting or delaying intestinal lipid absorption *via* a resin-like action and by increasing enterohepatic excretion of cholesterol in the bile acid (Yagarani *et al.,* 1992; Han *et al.,* 2001). Previous reports revealed that not only saponins but also flavonoids are also partially responsible for anti-obesity activity *via* lowering serum lipids by inhibiting the activity of HMG-CoA reductase and up-regulating the hepatic expression of peroxisome proliferators-alpha and gamma(Sharma *et al.,* 2008). Thus, the presence of hypolipidemic phytoconstituents like saponins, flavonoids and other phenolic compounds in aqueous and methanolic extract may be partially responsible for the anti-obesity efficacy of aqueous and methanolic stem bark extracts of *Cissus populnea*.

The increased in serum level of total cholesterol, triglyceride and LDL-cholesterol concentration in positive control group when compared with the normal control group is a clear evidence of coronary heart diseases since elevated serum level of total cholesterol, triglyceride and LDL-cholesterol are positively correlated with the incidence of coronary heart diseases, particularly atherosclerosis' (Snider *et al*., 2006 and Agrawal *et al*., 2009). Decrease in the serum level of HDL-cholesterol which could impact a corrective effect on the incidence of coronary heart disease (Feray, 2010), the significant decrease in the total cholesterol, triglyceride and LDL-cholesterol seems to suggest atherogenic potential for aqueous and methanolic stem bark extracts of *Cissus populnea*. The administration of different doses (100mg/kg and 200mg/kg body weight) of aqueous and methanolic stem bark extracts of *Cissus populnea* after exposure to poloxamer 407significantly reduced ($p \leq 0.05$) the concentration of total cholesterol, triglyceride and LDL-cholesterol when compared with untreated group (group2) and increase serum level of high density lipoprotein when compared with normal control. This showed that the extracts have the capacity to reduced cholesterol levels by improving the synthesis of HDL from the liver. Improving the synthesis of HDL from the liver could be as a result of regeneration of liver cells or mopping up of free radicals from the system. The result of this study is in line with the study of Joshua *et al., (2017).*

Triglyceride levels which are independent risk factors for cardiovascular problems (Wierzbicki and Mikhailidis, 2002) were fairly stable in both the low- and high-dose groups throughout the study period. Though not clearly significant, the triglyceride level decreased as a consequence of the extracts administered, confirming a hypolipaemic effect of the aqueous and methanolic extracts. This observation is supported by the report of Viana *et al*. (2004) that a closely-related species *Cissus sicyoides* has both hypoglycaemic and antilipaemic effects. It is also supported by the findings of another research group (Oben *et al*., 2006) that statistically significant reductions in weight and central obesity.

In hyperlipidemic conditions, there is an increase in plasma cholesterol, TAGs, LDL and decrease in HDL (Mitra *et al*., 1995). Under normal conditions insulin activates the enzyme lipoprotein lipase, which hydrolyzes triglycerides. However in diabetic state, lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridaemia. Alteration of serum lipid profile is known to occur in diabetes and this is likely to increase the risk of coronary heart disease (Al-Shamaony *et al*., 1994). Oral administration of aqueous and methanolic stem bark extracts of *Cissus populnea* produced significant beneficial effects in the lipid profile of the treated hyperlipidemic rats, significantly reducing total cholesterol, low density lipoprotein and triacylglycerol at higher dose whereas high density lipoprotein was significantly increased. The findings is close to the finding of Yusufoglu *et al*. (2015) that oral administration of *F. duranii* reversed the changes in plasma lipoproteins of diabetic rats and significantly improved their values towards near normal levels. These results suggest that the extracts might be considered as a substitute for drugs to reduce complications associated with hyperlipidemic conditions. Thus an excess fatty acid in the plasma produced by poloxamer 407 induced hyperlipidemia promotes the conversion of excess fatty acids into phospholipids and cholesterol in the liver. These two substances along with excess TAGs formed in the liver may be discharged from the liver in the form of lipoproteins. The HDL is an anti-atherogenic lipoprotein which transports cholesterol from peripheral tissues into the liver, thereby acting as a protective factor against coronary heart disease (Shirwaikar *et al*., 2004). It is well known that LDL plays an important role in artheriosclerosis and hypercholesterolemia. The decrease in cholesterol and LDL levels achieved by the administration of aqueous and methanolic stem bark extracts of *Cissus populnea* demonstrated a possible protection against hypercholesterolemia.

The influence of aqueous and methanolic stem bark extracts of *Cissus populnea* on normal albino rats were clearly expressed in the groups. The results suggests that aqueous and methanolic stem bark extracts of *Cissus populnea* had effect on the serum ALT, AST and ALP levels when compared with their controls. The results vary significantly, in aqueous and methanolic stem bark extracts of *Cissus populnea* groups as shown in table 2, the serum ALT, AST and ALP levels increased significantly ($P \leq 0.05$) in group II when compared with group I (normal control). These increments seen in table 2 were clear demonstration of cellular leakage and loss of functionality of membrane integrity (Saraswat *et al*., 1993). The presence of xenobiotics in the form of poloxamer 407 in experimental animals could cause derangement of biochemical processes (Uboh *et al*., 2010), increasing the activities of AST and ALT which are indicators of liver injuries (Edet *et al*., 2011). These injuries could have been caused by free radical

and peroxidants which are implicated in the pathogenesis of toxic liver injuries (Jalalpure *et al*., 2003). This finding is agree with the finding of Emeka and Obidoa, (2009) which showed the substantial presence of phytosterols in *G. latifolium* leaf extracts. Therefore, the metabolism of phytosterols could be the source of the free radical (Burns *et al*., 2000) which caused the compromise of the membranes of hepatocytes leading to leakage of enzymes and increases in the serum liver biomarkers. This reason may also be adduced for the significant serum AST increase in the experiment which is noticeable with most liver problems (Nuhu and Aliyu, 2008). The serum levels of aspartate transaminase, alanine transaminase and alkaline phosphatase at graded dose of the extracts groups decrease significantly ($P \geq 0.05$) when compared with the positive control. Decrease in in serum level of aspartate transaminase, alanine transaminase and alkaline phosphatase at graded dose of the extracts of *Cissus populnea* showed efficacy in ameliorating the liver damage. This could be due to the presence of bioactive phytochemicals that are well known as potent free radical scavengers (Mahadevan *et al*., 2009). This finding is in line with the finding of Odigie *et al*., (2003) and Adeyemi *et al*., (2014). This finding does not agree with the conclusion by Geidam *et al*., (2004) that the aqueous extract of this same plant from the northern part of Nigeria elevated alkaline phosphatase levels in both normal and diabetic rats. These increases were mostly statistically significant on group V and VII of ALT at the concentration of 200mg/kg when compared with normal control, but for the serum AST levels which was most at group V, VI and VII at concentration 100mg/kg and 200mg/kg when compared with normal control. The extract can therefore be said to be hepatoprotective and not hepatotoxic at graded doses.

The study investigated the antioxidant properties of aqueous and methanolic stem bark extracts of *Cissus populnea* and their effects on the antioxidant parameters in male albino rats. The high antioxidant capacity exhibited by the extracts at high and low concentrations could be ascribed to the synergistic action of the multi-antioxidant components of the extracts. The observed antioxidant effects suggest that individuals who consume food rich in the extracts could effectively reduce oxidative damage caused by oxidative stress resulting from free radicals generated in the body. It further indicates that individuals need not to consume excess of these extract before they can achieve the desired health benefits. The inhibition of production of the lipid peroxidation product (i.e., thiobarbituric acid reactive substances [TBARS]) in the presence of high and low concentrations of the extract could be due to the antioxidant activity of the bioactive components in the extracts. The extracts possess antioxidant potential capable of preventing lipid peroxidation and its associated adverse health effects. The increase TBARS concentration observed with increasing concentration of the extracts may be due to feedback inhibition of antioxidant activity of some of the antioxidant components at excess concentration and ability of some antioxidant components to function as pro-oxidants at higher concentrations. Although, these extracts possess high antioxidant activity at high and low concentrations, excessive consumption of these extracts could induce oxidative stress due to their ability to induce lipid peroxidation at higher concentrations.

For assessing antioxidant activity of aqueous and methanolic stem bark extracts of *Cissus populnea*, the oxidative stress was produced in rats at 300mg/kg dose of poloxamer 407 depending on the body weight of the animals and estimation of serum concentration of various antioxidants by standard biochemical methods. Serum levels of malondialdehyde, Superoxidedismutase and Catalase were measured. The results showed that poloxamer 407 increased serum malondialdehyde level and standard drug atorvastatin lowered serum malondialdehyde level in comparison with normal control. The graded extract doses decreased serum malondialdehyde levels. The decreased were statistically not significantly as compared to normal control. The significant ($p \leq 0.05$) increase in malondialdehyde concentrations observed in group II suggest that the richness of the induced drug in the animals in antioxidant components caused lipid peroxidation and oxidative stress resulting from reactive oxygen species/free radicals generated from normal metabolic reactions and leakages from incomplete transfer of electrons in electron transport chain. The significantly ($P \geq 0.05$) decreased serum levels of malondialdehyde concentrations observed in the administered aqueous and methanolic extracts indicate it's potentials on free radicals that could have been generated from respiratory burst and leakage from metabolic reactions causing lipid peroxidation in induced rats depicted by the elevated concentration of malondialdehyde a product of lipid peroxidation and damage caused to the cells by poloxamer 407 (Uroko *et al*., 2017).

Standard increased serum levels of the antioxidant enzymes (superoxide dismutase and catalase) significantly exerting a potent antioxidant effect. The reduction in the malondialdehyde concentration in the treatment group when compared with positive control(group II) could be attributed to induction of antioxidant enzymes activity like superoxide dismutase and presence of abundant non enzymatic antioxidants which mop up free radicals that may lead to increased lipid peroxidation (Halliwell, 2000 and Ikechukwu *et al*., 2015). The malondialdehyde concentration is not static in normal control rats rather it fluctuates base on prevailing physiological condition which could become problematic when there is excessive increment or deviation from the normal physiological range leading to damage of important cellular components and associated health consequences. Consumption of adequate plant extracts most especially the aqueous extract of *Cissus populnea* could confer some health benefits such as prevention of lipid peroxidation and oxidative stress caused by free radicals and thus prevent such consumers from suffering their adverse health consequences at adequate dose.

Malodialdehyde is a stable secondary aldehyde degeneration product of lipid peroxidation and is used as a biological marker for the assessment of lipid peroxidation.

An antioxidant works by retarding the process of oxidation by free radicals and further damage. Increased levels of measured antioxidant enzymes clearly pointed out the antioxidant potential of this plant both aqueous and methanolic extracts. Superoxide dismutase is an important endogenous antioxidant enzyme acting as the first line defense system against reactive oxygen species (ROS) which scavenges superoxide radicals to hydrogen peroxide and thus

provide protection against the deleterious effects of radicals (Olawale *et al*., 2008 and Mahantesh *et al*., 2012). hydrogen peroxide accumulated by this reaction leads to the formation of hydroxyl radicals which can be harmful too. Catalase works as antioxidant enzyme by virtue of scavenging these hydroxyl radicals by converting it rapidly to water and oxygen.

The induction of antioxidant enzymes activity (catalase and superoxide dismutase) by the plant extracts mopped up free radicals released from normal metabolic reactions and leakage of incompletely reduced oxygen from the electron transport chain in the mitochondria of the rats administered the extracts (Uroko *et al*., 2017).

The antioxidant effects observed in this study can be attributed to many phytochemicals in the experimental plant stem bark extracts as they are reported to possess the antioxidant activity. Among these, flavonoids have been very frequently correlated with the antioxidant potential of any plant extract. It has been proposed by Ye *et al*., 2012 that flavonoids have very strong capacity to eliminate free radicals in the blood and promotes the activities of antioxidant enzymes such as superoxide dismutase and catalase.

Antioxidant potential of the flavonoids has also been suggested by various other researchers in past (Fasuyi, 2005; Miladiyah *et al*., 2011;Noori, 2012). They are effective scavengers of various types of free radicals. Anthocyanins are also a group of flavonoids which can be helpful as antioxidants (Okeke, 2007 and Surush, *et al*., 2011). Gómez-Vásquez *et al.*, (2004) and Yi *et al*., (2011) reported the presence of ten important antioxidant substances in plants extract. Apart from these, the plants may show the presence of other constituents such as polyphenols, tannins, anthocyanins, alkaloids, glycosides, saponins, steroids, iron, and vitamins such as A, C, E which have been linked with the antioxidant potential by many researchers. The antioxidant activity demonstrated by both the aqueous and methanolic extracts could be attributed to the richness of the extracts in multi-antioxidant components such as β-carotene, flavonoids, total phenolics and vitamins A, E and C contents which prevent free radical damage, reducing risk of chronic diseases (Jamal *et al*., 2007 and Barcelos *et al*., 2015). These compounds carry out their antioxidant activities possibly by acting as reducing agents, chelating agents, hydrogen donors, or singlet oxygen quenchers. The antioxidant activity observed in this study is in-line with higher antioxidant activity of aqueous extracts of date palm reported by El-Nekeety *et al*. (2009). Thus, the consumption of dietary antioxidants from these extracts could be beneficial in preventing cardiovascular diseases, especially atherosclerosis normally associated with oxidative damage of biomolecules (Halliwell, 2002 and Jeon *et al*., 2002). Tsumbu *et al.*, (2011) also suggested the possible antioxidant activity of tannins. Ghani (1990) suggested the antioxidant potential of alkaloids, glycosides. Vitamins such as A, C, and E are very much known as antioxidants since long time as supported by various literature (Thomas, 2004; Okeke, 2007 and Noori, 2012). Thus, the antioxidant activity of aqueous and methanolic stem bark extracts of *Cissus populnea* can be explained on the basis of the presence of these antioxidant chemical constituents which are known to reduce oxidative stress by various mechanisms. Nassar *et al.*, (2013) have provided very strong correlation of flavonoids with analgesic, anti-inflammatory, antipyretic, antidiarrhoeal, antioxidant, and many other biological properties indicating the utmost importance of this phytoconstituents.

Consumption of food rich in components of aqueous extracts in the form of *Cissus populnea* and other similar foods could confer some protections against free radicals generated during oxidative stress and prevent some diseases and conditions such as diabetes, cancer, ageing and other related adverse health effects. Most extracts exhibit antioxidant activity via induction of gene expression of antioxidant enzymes as observed in the catalase and superoxide dismutase activity of the rats used in this study (Uroko, 2017). Synergistic action of non-enzymatic and enzymatic antioxidants (including endogenous and exogenous antioxidants) are required to fully combat or quench free radical reactions in the body as it has been shown that excessive production of free radicals such as superoxide radials under oxidative stress may inhibit the glutathione peroxidase activity, while excessive production of singlet oxygen, superoxide and peroxyl radical inhibits catalase activity (El-Nekeety *et al*., 2009). Oxidative stress damages cellular components and result in medical conditions most especially when the host antioxidants are not sufficient to quench oxidative reactions due to excess reactive oxygen and nitrogen species. Catalase and superoxide dismutase protect the body from reactive oxygen species like singlet oxygen ($O2^-$), hydroxyl ion (OH^-) and hydrogen peroxide (H_2O_2). It has been established that superoxide dismutase detoxifies superoxide radicals (O2–) to hydrogen peroxide (H_2O_2) whereas, glutathione peroxidase reduces hydrogen peroxide to non toxic products (Liu, 2010). Excessive generation of reactive oxygen and nitrogen species could lead to depletion of antioxidant enzymes. Thus, agents that possess ability to induce the expression of genes coding for antioxidant enzyme and increase the concentrations and activities of antioxidant enzymes as seen in this study are highly valuable in ameliorating the effects of excess oxidants in the body. Consumption of adequate amount of nutrients known to possess significant antioxidant activity like *Cissus populnea* are required to maintain a healthy life.

VII. CONCLUSION

The findings of this study have demonstrated that aqueous and methanolic stem bark extracts of *Cissus populnea* possess anti-hyperlipidemic effect and can effectively maintained the lipid profile within acceptable level. This suggests that the extract could be efficiently used to treat cardiovascular disease and prevent most of the health complications associated with the condition. This revealed that aqueous and methanolic stem bark extracts of *Cissus populnea* have the ability to protect the liver against injury, reduce blood cholesterol levels the leading cause of hyperlipidemia. This study shows that the aqueous and methanolic extracts of *Cissus populnea* possess significant increase in antioxidant activities which could help in ameliorating the effects of oxidative stress resulting from free radicals from both endogenous and exogenous sources.

REFERENCES

[1] Adeyemi, S. B., Ogundele, K. O. and Animasaun, M. A. (2014). Influence of drying methods on the proximate and phytochemical composition of Moringa oleifera Lann. Global journal of medicinal plant research, 2 (1) 1-5.
[2] Agrawal, A., Houghton, L.A. and Morit, S. (2009). Clinical Trail: The Effects of a Fermented Milk Product Containing Bifidobacterium Lactis DN -173-010 on Abdominal Distension and Gastrointestinal transit in irritable bowel syndrome with constipation. Alimentary Pharmacology and Therapeutics, 29: 104-114.
[3] Al-Shamaony, L., S. M. Al-Khazraji and H. A. A. Twaiji, (1994). Hypoglycaemic effect of Artemisia herba alba. II. Effect of a valuable extract on some blood parameters in diabetic animals. Journal of Ethnopharmacology, 43: 167-171.
[4] Amador, E. and Wacker, W. (1962). Serum Glutamic Oxaloacetic Transaminase Activity. A new Modification and an Analytical Assessment of Current Assay. Technics. Clinical Chemistry.
[5] Asheneni, V. D. (2014). Anti-hyperlipidemic potential of Vitex doniana ethanolic extracts on poloxamer 407 induced hyperlipidemic and normal rats. Faculty of Science (Biochemistry). Ahmadu Bello University, Zaria.
[6] Barcelos, E. S., de Almeida Rios, R. N., Cunha, R. L. and Motoike, S.Y. (2015). Oil palm natural diversity and the potential for yield improvement. Frontier in Plant Science, 6. 10.3389/fpls.00190.
[7] Bierman, E. L. and Brunzell, J. D. (1978). Interrelation of atherosclerosis, abnormal lipid metabolism, and diabetes mellitus. In: Ktzen HM, Mahler I, eds. Advances in modern nutrition, Vol. 7. New York: Wiley,:187-210.
[8] Burkill, H. M. (2000). The Useful Plants of West Tropical Africa, vol. 5. Families S-Z, pp.296-297. Royal Botanic Gardens, Kew.
[9] Burns, D., Renolds, W.F., Buchanan, G., Reese P.B. and Enriquiez, R.G. (2000). Assignment of 23C spectra and investigation of hindered side-chain rotation in lupeol derivatives. Journal of Magnetic Resonance in Chemistry, 387:488-493.
[10] Cordell, G. A. and Colvard, M. D. (2012). Natural products and traditional medicine: turning on a paradigm. Journal of Natural Products, 75: 514–525.
[11] Edet, E. E., Atangwho, I. J., Akpanabiatu, M. J., Edet, T. E., Uboh, F. E and David-Oku, M. (2011). Effect of Gongronema latifolium leaf extract on some liver enzymes and protein levels in diabetic and non-diabetic rats. Journal of Pharmacuetical and Biomedical Science, 1: 104-107.
[12] El-Nekeety, A. A., El-Kady, A. A. Soliman, M. S. Hassan, N. S. and Abdel-Wahhab, M. A. (2009). Protective effect of Aquilegia vulgaris (L.) against lead acetate-induced oxidative stress in rats. Food and Chemical Toxicology, 47: 2209-2215.
[13] Emeka, E. J. I. and Obidoa, O. (2009). Effect of a long term consumption of a diet supplemented with leaves of Gongronema latifolium Benth. on some biochemical and histological parameters in male albino rats. Journal Biological Science,; 9: 859-865.
[14] Fasuyi, A.O. (2005) Nutrient composition and processing effects on cassava Leaf (Manihot esculenta, Crantz) antinutrients. Pakistan Journal of Nutrition,4:37–42.
[15] Feray, C. (2010). Towards predicting the therapeutic response in patients with hepatitis C. International Journal of Gastroenterology and Hepatology, vol 31 (2): 339-343.
[16] Friedwald W. T. (1972). Methods for the determination of LDLCholesterol.Clinical Chemistry, 18:499-502.
[17] Galal, R. M., Zaki, H. F., Seif El-Nasr, M. M. and Agha, A. M. (2012). Potential protective effect of honey against paracetamol-induced hepatotoxicity. Archive of Iranian Medicine,15:674–80.
[18] Geidam, M. A., Adoga, G. I. and Sanda, F. A. (2004). Effects of aqueous stem bark extract of Cissus populnea on some serum enzymes in normal and alloxan-induced diabetic rats. Pakistan Journal of Biological Sciences, 7(8): 1427 – 1429.
[19] Ghani, A. (1990). Introduction to Pharmacognosy. 45. Zaria, Nigeria: Ahmadu Bello University Press, Ltd. pp. 187–97.
[20] Gill, L.S., (1992). Ethno medicinal uses of plant in Nigeria. University of benin Press Nigeria, pp. 212.
[21] Gómez-Vásquez, R., Day, R., Buschmann, H., Randles, S., Beeching, J.R. and Cooper, R.M.(2004). Phenylpropanoids, phenylalanine ammonia lyase and peroxidases in elicitor-challengedcassava (Manihot esculenta) suspension cells and leaves. Annuals of Botany, 94:87–97.
[22] Halliwell, B. (2000). Lipid peroxidation, antioxidants and cardiovascular disease: How should we move forward? Cardiovascular Research journal; 47: 410-418.
[23] Halliwell, B., (2002). Effect of diet on cancer development: Is oxidative DNA damage a biomarker? Free Radical Biology and Medicine, 32: 968-974.
[24] Han, L.K., Kimura, Y., Kawashima, M., Takaku, T., Taniyama, T., Hayashi, T., Zheng, Y.N. and Okuda, H. (2001). Anti-obesity effects in rodents of dietary tea saponin, a lipase inhibitor. International journal Obesity and Related Metabolic Disorder, 25, 1459–1464.
[25] Haussament T.U. (1977). Quantitative determination of serum alkaline phosphatase.Clinica Chimica Acta,35: 271-273.
[26] Hetal, R. C. and Dion, R. B. (2013). The Single Dose Poloxamer 407 Model of Hyperlipidemia; Systemic effects on Lipids assessed using Pharmacokinetic Methods, and Its effects on Adipokines. Faculty of Pharmacy and Pharmaceutical Sciences, 3142H Katz Group Centre for Pharmacy and Health Research, University of Alberta, Edmonton, Canada.
[27] Ikechukwu, U.R., Adeyi, S.R.S., Hadiza, M.K., and Lilian, A.C. (2015). Effect of methanol extract of Abrus precatorius leaves on male wistar albino rats induced liver damage using carbon tetrachloride (CCl4). Journal of Biological Science, 15: 116-123.
[28] Jalalpure, S.S., Patil, M.B., Prakash, N.S., Hemalata, K. and Manvi, F.V. (2003). Hepatoprotective activity of fruits of Piper longum L. Indian Journal of Pharmacuetical Sciences 65: 363-366.
[29] Jamal, P., Alam, M. Z. and Mohamad, A. B. (2007). Microbial bioconversion of palm oil mill effluent to citric

acid with optimum process conditions. Proceedings of the 3rd Kuala Lumpur International Conference on Biomedical Engineering, Kuala Lumpur, Malaysia, pp: 483-487.
[30] Jeon, S. M., Bok, S. H., Jang, M. K., Kim, Y. H. and Nam, K.T. (2002). Comparison of antioxidant effects of naringin and probucol in cholesterol-fed rabbits. Clinical Chemistry and Biological Science, 317: 181-190.
[31] Joshua, P.E., Ukegbe, C.Y., Eze, O., Nkwocha, C.C., Okafor, J.O., Amaechi, O. Nduka, F.O., Ozougwo, V., Eyenike, J.O. and Ugochukwu, N.E. (2017). Biochemical Effects of the Aqueous Extract of Hibiscus sabdariffa on Liver Marker Enzymes and Lipid Profiles in Acetaminophen-challenged Rats. Research Journal of MedicinalPlants,11:48-54.
[32] Kone, W. M., Traore, D. and Dosso, D. (2005). Journal of Ethnopharmacol. pp.43-49.
[33] Liu, C. M., Zheng, Y.L., Lu, J., Zhang, Z. F., Fan, S. H., Wu, D. M. and Ma, J. Q. (2010). Quercetin protects rat liver against lead-induced oxidative stress and apoptosis. Environmental Toxicology and Pharmacology, 29: 158-166.
[34] Mahadevan, N., Shivali, A. and Kamboj, P. (2009). Hibiscus sabdariffa Linn: an overview. Avicenna journal of phytomedicine; 8:77-83.
[35] Mahantesh, S. P., Gangawane, A. K. and Patil, C. S. (2012). Free radicals, antioxidants, diseases and phytomedicines in human health: Future perspects. World Research Journal of Medicine and Aromatic Plants;1:6–10.
[36] Marklund, S. and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry, 47:469-474.
[37] Megallic S., Aktan F., Davies N.M. and Roufogalis B.D. (2005).Phytopreventative anti hyperlipidemic effects of Gynostemma pentaphyllum in rats. Journal of Pharmaceutical Sciences, 8(3): 507-515.
[38] Miladiyah, I., Dayi, F. and Desrini, S. (2011) Analgesic activity of ethanolic extract of Manihot esculenta Crantz leaves in mice. University Medical center, 30:3–10.
[39] Mitra, S.K.S., Gopumadhavan, T.S., Muralidhar, S.D., Anturlikar and Sujatha, M.B. (1995). Effect of D-400, a herbomineral preparation on lipid profile, glycated haemoglobin and glucose tolerance in streptozotocin induced diabetes in rats. Indian Journal of Experimental Biology, 33:798-800.
[40] Nassar, M.I., Aboutabl, E.S.A., Eskander, D.M., Grace, M.H., El-Khrisy, E.D. and Sleem, A.A. (2013). Flavonoid glycosides and pharmacological activity of Amphilophiu paniculatum. Pharmacognosy Reserch,5:17–21.
[41] N.C.E.P.W.G. (1995). National Cholesterol Education Program Working Group on Lipoprotein Measurement. Recommendation on lipoprotein Measurement. NHLBI, NIH Publication No. 95-3044, Bethesda.
[42] Noori, S. (2012). An overview of oxidative stress and antioxidant defense system. Scientific Report, 1:413.
[43] Nuhu, A.A. and Aliyu, R. (2008). Effects of Cassia occidentalis aqueous leaf extract on biochemical markers of tissue damage in rats. Tropical Journal of Pharmaceutical Research, 7: 1137-1142.
[44] Oben, J., Kuate, D., Gabriel, Agbor, G., Momo, C. and Talla, X. (2006). The use of a Cissus quadrangularis formulation in the management of weight loss and metabolic syndrome. Lipids in Health and Dis., 5: 24.
[45] Odigie, I. P. Ettarh R. R. and Adigun S. A. (2003). Chronic administration of aqueous extracts of Hibiscus sabdariffa attenuate hypertension and reverses cardiac hypertrophy in 2K-1C hypertensive rats. Journal of ethnopharmacology; 6:102-110.
[46] Ojekale, A. B., Ojiako, O. A., Saibu, G. M., Lala, A. and Olodude, O. A. (2006). Long term effects of aqueous stem bark extract of Cissus populnea (Guill. and Per.) on some biochemical parameters in normal rabbits. African Journal of Biotechnology, Pp 250-252.
[47] Okeke, C.U. and Iweala, E. (2007). Antioxidant profile of Dioscorea rotundata, Manihot esculenta,Ipoemea batatas, Vernonia amygdalina and Aloe vera. Research Journal of Medicinal Plants, 4:4–10.
[48] Olawale, O., Ikechukwu, N.E., Grace, T.O. and Chidiebere, E.U. (2008). Oxidative stress and superoxide dismutase activity in brain of rats fed with diet containing permethrin. Biokemistri.;20:93–98.
[49] Salahdeen, H. M. and Yemitan, O. K. (2006). Neuropharmacological effects of aqueous leaf extract of Bryophyllum pinnatum in mice. African Journal of Biomedical Research; 9:101-7.
[50] Saraswat, B., Visen, P.K., Patnaik, G.K. and Dhawan, B.N. (1993). Anticholestic effect of picroliv, active hepatoprotective principle of Picrorhiza kurrooa, against carbon tetrachloride induced cholestasis. Indian Journal of Experimental Biology; 31: 316-318.
[51] Sharma, B., Balomajumder, C. and Roy, P. (2008). Hypoglycaemic and hypolipidaemic effects of flavonoid rich extract from Eugenia jambolana seeds on streptozotocin induced diabetic rats. Food Chemical and Toxicology; 46: 2376–2383.
[52] Shirwaikar, A., Rajendran, K., Kumar, C.D. and Bodla, R. (2004). Antidiabetic activity of aqueous leaf extract of Annona squamosa in streptozotocin-nicotinamide type 2 diabetic rats. Journal of Ethnopharmacology; 91: 171-175.
[53] Slater, T.F. and Sawyer, B.C. (1971). The stimulatory effects of carbon tetrachloride and other halogenoalkanes on peroxidative reactions in rat liver fractions in vitro. General features of the systems used. Journal of Biocheistry.;123:805–814.
[54] Snider, M. D., McGarry, J. D. and Hanson, R. W. (2006). Lipid Metabolism 1: Synthesis, torage and utilization of Fatty Acids and Triacylglycerols In: Thomas M. Delvin, editor. Textbook of Biochemistry with Clinical correlations (6th edition). Wiley-Liss Publication, USA. P. 661-693.
[55] Soladoye, M. O. & Chukwuma, E.C. (2012). Quantitative phyto¬chemical profile of the leaves of Cissus populnea Guill. & Perr. (Vitaceae) – an important medicinal plant in Central Nigeria. – Archive of Applied Science Research; 4(1): 200-206.
[56] Stein E.A. (1987). Lipids, lipoproteins and Apolipoproteins metabolism.Fundamentals of Clinical

Chemistry.3rd edition, W.B Saunders Company. Philadelphia, 470-479.
[57] Suresh, R., Saravanakumar, M. and Suganyadev, P.(2011). Anthocyanins from Indian cassava (Manihot esculenta Crantz) and its antioxidant properties. International Journal of Pharmcuetical Science Research;2:1819–28.
[58] Thomas, D.R. (2004). Vitamins in health and aging. Clin Geriatr Med.;20:259–74.
[59] Trease, G. E. and Evans, M.C. (2005). Pharmagnosy 14th Edn. Elsevier, New delhi, India,pp. 431-452.
[60] Tietz N. W. (1990). Clinical guide to laboratory tests. In: Fundamentals of Clinical Chemistry (ed N.W.Tietz), W.B Saunders, Philadelphia.
[61] Tsumbu, C.N., Deby-Dupont, G., Tits, M., Angenot, L., Franck, T., Serteyn, D. (2011). Antioxidantand antiradical activities of Manihot esculenta Crantz (Euphorbiaceae) leaves and other selected tropical green vegetables investigated on lipoperoxidation and phorbol-12-myristate-13-acetate (PMA) activated monocytes. Nutrients.;3:818–38.
[62] Uboh, F.E., I.E. Okon and M.B. Ekong, (2010). Effect of aqueous extract of Psidium guajava leaves on liver enzymes, histological integrity and hematological indices in rats. Gastroenterology Research; 3: 32-38.
[63] Uroko, R. I., Agbafor, A., Uchenna, O. N., Achi, N. K., Egba, S. I., Nweje-Anyalowu, P. C. and Ngwu, O. R. (2017) Evaluation of Antioxidant Activity of Aqueous Extracts of Palm Fruits (Elaeis guineensis). Asian Journal of Biochemistry; 12 (2): 49-58.
[64] Viana, G.S.B., Medeiros, A.C., Lacerda, A.M., Leal, K.A.M., Vale, T.G. and de Abreu Matus, F.J.(2004). Hypoglycemic and anti-lipemic effects of the aqueous extract of Cissus sicyoides. BMC Pharmacology; 4: 9.
[65] Wacnic, R.G. and Alber J.J. (1978).A comprehensive evaluation of the heparin manganese precipitation procedure for estimating high density lipoprotein cholesterol. Journal of Lipid Research, 19: 65-76.
[66] Welkowitz, J. (1976). Introductory statistics for the behavioral sciences. Academic press.
[67] WHO. (2002). Traditional Medicinen and Alternative Medicines. Geneva.; Fact Sheet. No. 271.
[68] Wierzbicki, A. S. and Mikhailidis, D. P. (2002). Beyond LDL-C – the importance of raising HDL-C, Current Med Res Opin. 18(1): 36 – 44.
[69] Ye, Y., Guo, Y. and Luo, Y.T. (2012). Anti-inflammatory and analgesic activities of a novel biflavonoid from shells of Camellia oleifera. International Journal of Molecular Science;13:12401–11.
[70] Yi, B., Hu, L., Mei, W., Zhou, K., Wang, H., Luo, Y., (2011). Antioxidant phenolic Compounds of cassava (Manihot esculenta) from Hainan. Molecules;16:10157-67.
[71] Yusufoglu, H.S., Soliman, G.A., AbdelRahman, R.F., Abdel Kader, M.S. and Ganaie, M.A. (2015). Antihyperglyce mic and antihyperlipidemic effects of Ferula duranii in experimental type 2 diabetic rats. International Journal of Pharmacology; 11: 532-541.
[72] Yugarani, T., Tan, B.K., The, M. and Das, N.P. (1992). Effect of polyphenolic natural products on the lipid profiles of rats fed high-fat diets. Lipids; 27: 181–186.