ejpmr, 2015,2(2), 76-94

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH
www.ejpmr.com

Research Article
ISSN 2394-3211
EJPMR

EFFECT OF METHANOL EXTRACT OF *DIALIUM GUINEENSE* LEAVES ON LIVER CELL DAMAGE AND LIPID PROFILE OF ALBINO RATS

Aloh,G.S.[1], Obeagu, Emmanuel Ifeanyi[2], Odo Christian Emeka[1], Kanu Stella Ngozika[3], Okpara, kingsley Ezechukwu[4], Nwosu, D.C.[5], Ugwu Getrude Uzoma[6] and Ezeumudo Oluebube C[1]

[1]Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

[2]Diagnostic Laboratory Unit,University Health Services, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

[3]Abia State University Teaching Hospital, Aba, Abia State, Nigeria.

[4]Rivers State College of Health Technology, Port Harcourt.

[5]Department of Medical laboratory Science, Imo State University, Owerri, Nigeria.

[6]School of Nursing Science, ESUT Teaching Hospital, Parklane, Enugu, Nigeria.

Article Received on 29/12/2014 **Article Revised on 21/01/2015** **Article Accepted on 12/02/2015**

***Correspondence for Author**
Obeagu, Emmanuel Ifeanyi
Diagnostic Laboratory Unit,University Health Services, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
Phone: +2348037369912.
emmanulobeagu@yahoo.com
obeagu.emmanuel@mouau.edu.ng

ABSTRACT

This study investigated the protective effect of oral administration of the different doses (250mg and 500mg) of methanol extract of *Dialium guineense* leaves against liver cell damage and lipid profile of albino wistar female rats. Analysis of the following parameters were made: alanine aminotransferase (ALT), aspartate aminotransferase (AST), Superoxide dismutase (SOD), catalase, glutathione (GSH), malondialdehyde (MDA), vitamin C (VIT.C), vitamin E (VIT.E), high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (T.CHOL), total protein (T.PROT), triacylglycerides (TAG) & albumin (ALB). Sixteen (16) albino rats were distributed into four (4) groups. Group 1(Normal control) was not subjected to hepatocyte damage, but only received normal feed and water *ad libitum*. Group 2 (negative control) received the 2.5ml of $C_6H_{14}/CHCl_3$ (hepatotoxicant) but was not given the extract. Groups 3 and 4 were given the $C_6H_{14}/CHCl_3$ and were treated with 250mg and 500mg/kg

body weight extract of *Dialium guineense* respectively. The results indicate that the 250mg and 500mg of the extract caused elevation in total cholesterol and high-density lipoprotein significantly (P<0.05) when compared with the negative control. 250mg of extract on low density lipoprotein also increased significantly (p<0.05) in comparison with the negative control. Similarly, the effect of 250mg and 500mg of the extract on vitamin E and catalase was significantly elevated (p<0.05), whereas 500mg only caused vitamin C and SOD to increase significantly when compared with the negative control. The effect of 500mg extract on ALT and AST has significant elevation (p<0.05) when compared with the negative control. No significant difference was observed in the other parameters in comparison with the normal and control. Therefore, methanol extract of *Dialium guineense* did not exert a hepatoprotective effect in the rats used, and caused the unusual effect of causing cholesterol, HDL and LDL elevations.

KEYWORDS: Metanol extracts, *Dialium guineesnse* leaves, liver function parameters, Lipid profile, Albino Rats.

INTRODUCTION

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is the largest organ of the human body weighing approximately 1500 g, and is located in the upper right corner of the abdomen on top of the stomach (Naruse *et al*., 2007), right kidney and intestines and beneath the diaphragm. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction (Sharma *et al.*, 1991). The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for the overall health and well being (Subramaniam and Pushpangadan, 1999). Liver parenchyma serves as a storage organ for several products like glycogen, fat and fat soluble vitamins. It is also involved in the production of a substance called bile that is excreted to the intestinal tract. Bile aids in the removal of toxic substances and serves as a filter that separates out harmful substances from the bloodstream and excretes them. (Saukkonen *et al*., 2006).

BACKGROUND TO THE STUDY

Hepatotoxicity implies chemical-driven liver damage. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Hepatotoxicants are exogeneous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals, Natural chemicals, herbal

remedies and dietary supplements. Hepatotoxicity may result not only from direct toxicity of the primary compound but also from a reactive metabolite or from an immunologically-mediated response affecting hepatocytes, biliary epithelial cells and /or liver vasculature (Saukkonen *et al*., 2006; Deng *et al*., 2009). More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures. More than 75 percent of cases of idiosyncratic drug reactions result in liver transplantation or death (Ostapowicz *et al.,* 2002).

The hepatotoxic response elicited by a chemical agent depends on the concentration of the toxicant which may be either parent compound or toxic metabolite, differential expression of enzymes and concentration gradient of cofactors in blood across the acinus (Kedderis, 1996). Hepatotoxic response is expressed in the form of characteristic patterns of cytolethality in specific zones of the acinus. Hepatotoxicity related symptoms may include a jaundice or icterus appearance causing yellowing of the skin, eyes and mucous membranes due to high level of bilirubin in the extracellular fluid, pruritus, severe abdominal pain, nausea or vomiting, weakness, severe fatigue, continuous bleeding, skin rashes, generalized itching, swelling of the feet and/or legs, abnormal and rapid weight gain in a short period of time, dark urine and light colored stool (Bleibel *et al*.,2007; Chang *et al*., 2003).

STATEMENT OF THE PROBLEM

Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to drugs or chemicals or their reactive metabolites (hepatotoxicants). The distinction between injury and function is important because it is mainly when function is impaired that symptoms and clinical significant diseases follow. Many research works have been done on *Dialium guineense* but its hepatoprotective properties and lipid profile have not been reported. In this work, *Dialium guineense* is evaluated for its lipid profile activity and hepatoprotective effect.

OBJECTIVES OF THE STUDY

- To determine the hepatoprotective potentials of the methanolic extract of the leaves of *Dialium guineense.*
- To determine the total antioxidant activity of the leaves of *Dialium guineense*.

- To determine the total proteins, albumin, and the lipid profile of the leaves of *Dialium guineense.*

SIGNIFICANCE OF THE STUDY

The significance of this study is to provide a hepatoprotective effect of the leaves of Dialium guineense on the Albino wistar female rats subjected to hepatocyte damage.

MATERIALS

- **Source of chemicals**: All the chemicals and reagents used were of analytical grade.
- **Collection of plant samples:** The fresh leaves of Dialium guineense was collected from the university compound, Michael Okpara University of Agriculture, Umudike. The plant was identified by Dr Godwin Aloh of the department of Biochemistry and was authenticated by Mr Udoka of Forestry department, College of Natural and Environmental Management, Michael Okpara University of Agriculture Umudike Umuahia Abia State, Nigeria. The leaves were disstocked, ground to powder using a grinding machine and was dissolved with methanol and allowed to stand for 48hrs. It was then filtered using Whatman filter paper, and then the filtrate was evaporated to dryness using water bath at 60^{0c}. The dried extract was stored until use for the experiment. During administration, the crude extract (1.6g) was dissolved in 100mls of deionized water and then administered orally to the female albino wistar rats with mean weight of 0.127kg.

- **The experimental animals**: Female albino wistar rats with mean weight of 0.127kg were used for this study. The animals were obtained from the animal house of the department of zoology, University of Nsukka, Enugu State. The animals were bred and housed in the animal house of the department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike Abia State, Nigeria. Animals were randomly distributed in clean and standard cages with a good ventilation and 12h light/dark cycle. They were provided with standard rat's pellets (Pfizer Livestock feeds plc, Abia State, Nigeria) and water ad libitum. The research was conduted in accordance with the internationally accepted principles for laboratory animal use and care, as found in for example the European community guidelines (ECC Directive of 1986;86/609/ECC).

- **Design of experiment**: In this experiment, sixteen rats were randomly divided into 4 groups of 4 animals each. In these 4 groups, group 1(normal control) which were not induced any hepatotoxicant received normal feed and water. Group 2(Negative control) was subjected

to hepatotoxicity but were left untreated. Group 3(test group) and Group 4(test group) were given orally with the methanolic extract of the leaves of *Dialium guineense* at doses of 250mg and 500mg respectively.

- **Induction of Hepatotoxicant**: The female albino wistar rats were induced by an oral dose of 2.5ml stringe of the combination of chloroform and hexane preparation. Low appetite for food and water, reduction in activity and weight reduction was noticed.

- **Statistical analysis**: The values were analyzed statistically using statistical package for social sciences (SPSS) (Version 17.0). The data were expressed as mean± Standard Deviation using bar charts. Comparisons were made between the controls and the test groups fed with the extract of *Dialium guineense* using paired t- test. The significance in difference was accepted at $p<0.005$.

METHODS

ALANINE AMINOTRANSFERASE (ALT) ASSAY

Principle: Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

$$\alpha\text{- Oxoglutarate + L-alanine} \xrightarrow{\text{GPT}} \text{L-glutamate + Pyruvate}$$

Procedure: The method of Reitman and frankel (1927) was adopted. Reagent blank was prepared by measuring 0.5ml buffer into a test tube and 0.1ml of distilled water was added, and allowed to incubate for 20mins. 0.5ml of 2, 4 dinitrophenylhydrazine was then added and allowed to stand for 20mins. 5.0ml of Na0H was also added, mixed and incubated for 30mins and the absorbance read. The sample was prepared by pipetting 0.1ml of sample into a test tube and 0.5ml of reagent buffer was added and allowed to incubate for 30mins at 37^{0c}. 0.5ml of 2, 4 dinitrophenylhydrazine was then added and allowed to stand for 20min at $25^{0c.}$. 5.0ml was finally added and the absorbance of sample (A_{sample}) was read against reagent blank after 5mins.

ASPARTATE AMINOTRANSFERASE (AST) ASSAY

Principle: AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

$$\alpha\text{- Oxoglutarate + L-aspartate} \xrightarrow{\text{GOT}} \text{L-glutamate + Oxaloacetate}$$

Procedure: The method used here was the same method adopted by Reitman and Frankel (1957) described in ALT above.

DETERMINATION OF ALBUMIN

Albumin is the most abundant serum protein representing 55-65% of the total protein. It is synthesized in the liver and has a half life of 2 to 3 weeks.

Principle: The measurement of serum albumin is based on its quantitative binding to the indicator 3, 3′, 5, 5′ – tetrabromo –M cresol Sulphonephthalein (bromocresol green, BCG). The albumin-BCG-Complex absorbs maximally at 578nm, the absorbance being directly proportional to the concentration of albumin in the sample.

Procedure: The method of Grant et al., (1987) was adopted. The reagent blank (So), standard (S_1) and sample were prepared by mixing 3µl of distilled water (ddH_20) with 1000µl of reagent respectively. It was then incubated for 20mins at 20-25^{0c} and the absorbance taken within 60mins. In different test tubes, 0.01ml of standard (CAL) was mixed with 3.00ml of BCG reagent and allowed to incubate for 5 minutes at 25^{0c}. The reagent blank and the sample also took the same procedure after which the absorbance of the sample (A sample) and of the standard (A standard) against the reagent blank were measured.

The albumin concentration in the sample may be calculated from the following formula:

Albumin concentration (g/dl) = $\frac{A_{sample}}{A_{standard}} \times$ Concentration of standard

TOTAL PROTEIN ASSAY

Principle: At alkaline pH value, proteins form a stable complex with Cu (11) ions which is photometrically measured.

Procedure: Standard solution was prepared by measuring 0.02ml of standard in a cuvette and mixed with water and allowed to stand for 10mins. The sample test was also prepared using the same procedure. Their absorbance read against a blank of 1ml of reagent at 540nm.

$$\frac{\text{Sample O.D}}{\text{Standard O.D}} \times 5 - \text{g of proteins/dl} = \text{Total serum proteins}$$

DETERMINATION OF CHOLESTEROL IN SERUM

Cholesterol measurements are used in the diagnosis and treatments of lipid lipoprotein metabolism disorders.

Principle: The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

Procedure: Serum was diluted with water in the ratio of 1:19 and cholesterol standard diluted with glacial acetic acid. Tree test tubes were set up as follows:

	Test	Standard	Blank
Ferric Chloride reagent	5.0ml	5.0ml	5.0ml
Dilute Serum	0.5ml	-	-
Dilute Standard	-	0.5ml	-
Distilled Water	-	-	0.5ml

The test tubes where shaken for 10 seconds to mix the content of each tube. The tubes were then placed in a boiling water bath for exactly 90 seconds and cooled in a running tap for 5mins. The absorbance was then read against the blank at 560nm.

$$\text{Serum Cholesterol (mg/dl)} = \frac{\text{Ab Test}}{\text{Ab Standard}} \times 250$$

Serum Total Cholesterol (mMol/l) = mg/dl x 0.0259

DETERMINATION OF TRIACYGLYCERIDES IN SERUM

Triglyceride measurements are used in the diagnosis and treatment of diseases involving lipid metabolism and various endocrine disorders e.g diabetes mellitus, nephrosis and liver obstruction.

Principle: The principle of this test includes the following:

1. Triglycerides + $H_20 \xrightarrow{\text{Lipases}}$ Glycerol +Fatty acids
2. Glycerol-3-phosphate + $0_2 \xrightarrow{\text{GPO}}$ Dihydroxyacetone + Phosphate + H_20_2
3. Glycerol + ATP $\xrightarrow{\text{GK}}$ Glycerol-3-phosphate + ADP
4. $2H_20$ + 4-aminophenazone + 4 Chlorephenol $\xrightarrow{\text{POD}}$ Quinoneimine + Hcl + $4H_20$

Procedure: Tree test tubes were set as follows

	Test	Standard	Blank
Colour Reagent	3.0ml	3.0ml	-
Serum	0.03ml	-	-
Titre Standard	-	0.03ml	-
Distilled Water	-	-	0.03ml

They were mixed and incubated at 37^{0c} for 15mins. The absorbance of test and standard was read against the blank at 420nm.

Serum Triglycerides (mg/dl) = $\frac{\text{Ab Test}}{\text{Ab Standard}} \times 100$

Serum triglycerides mMol/L = mg/dl x 0.0113

DETERMINATION OF HDL-CHOLESTEROL IN SERUM

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

Procedure: 1ml of serum was pipette into a centrifuge tube. 0.1ml of PTA reagent was added and mixed. 0.005ml of magnesium Chloride was then added and mixed. It was centrifuged at 2500rpm or 1500g for 30mins. The supernatant was removed with a Pasteur pipette. Then, the cholesterol level in the supernatant was estimated by diluting with distilled water and read in a spectrophotometer. Cholesterol standard was also diluted with glacial acetic acid and the absorbance read.

Serum HDL cholesterol (mg/dl) = $\frac{\text{Ab Test}}{\text{Ab Standard}} \times 115$

Serum HDL Choleterol (mmol/l) = mg/dl x 0.0257

DETERMINATION OF LDL-CHOLESTEROL IN SERUM:

Principle: LDL-cholesterol can be determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethylene-glycolmonomethyl ether.

Procedure

1. Precipitation reaction: The precipitant solution (0.1ml (3 drops) and the sample (0.2ml) was mixed well and allowed to stand for 15min. approximately at room temperature (20-25^{0c}). It was centrifuged at 2,000xg/15min and the cholesterol concentration in the supernatant was determined.
2. Cholesterol assay: The concentration of the serum total cholesterol according to the QCACHOD-PAP method was determined.

LDL-Cholesterol (mg/dl) = Total Cholesterol(mg/dl) – 1.5 x supernatant cholesterol (mg/dl)

LIPID PEROXIDATION (MALONDIALDEHYDE MDA) ASSAY

Lipid peroxidation was determined spectrophotometrically by measuring the level of the lipid peroxidation product, malondialdehyde (MDA).

Principle: Malondialdehye (MDA) reacts with thiobarbituric acid to form a red or pink colour complex which in acid solution, absorbs maximally at 532nm.

$$\text{MDA} + 2\text{TBA} \longrightarrow \text{MDA: TBA}_{\text{adduct}} + H_20$$

Procedure: 0.1ml of the serum was added into 0.9mls of H_20. 0.5ml of 45% TCA was added. 0.5mls of 1% TBA in 0.8% Na0H was also added. It was boiled for 40mins and cooled. It was then mixed with 0.1ml of 20% SDS (Sodium dedocyl sulphate) and the absorbance at 532nm and 600nm was taken.

ASCORBIC ACID (VITAMIN C) ASSAY

Principle: Ascorbic acid is oxidized and converted to diketoglutamic acid in strong acid solution and a diphenyl hydrazone by reacting with 2,4 dinitrophenyl hydrazone. The hydrazone dissolves into a strong sulphuric acid solution to produce a red colour which can be maeasure colorimetrically at wavelength of 1500nm.

Procedure: 1.0ml of plasma was placed into a small test tube. 1.0ml of 10% trichloroacetic acid and 0.5ml of chloroform was also added. It was stoppered, shaken vigorously up to 15 seconds and centrifuged at 3,000rpm. 1.0ml of clear supernatant was placed into a test tube. Blank and standard was prepared by adding 0.5ml of 10% trichloroacetic acid to 0.5ml of water and working standard. To each tube was added 0.4ml of prepared combined colour reagents. The tubes were stoppered and placed to a water bath at 56^{0C} for 1 hour. They were cooled in the ice bath for about 5 minutes. Each tube was added, slowly with mixing 2.0ml of

ice cold 85% sulphuric acid. The tubes were left at room temperature for 30 minutes, then mixed and the optical density (OD) read in the colorimeter at 490nm using blank to zero the instrument. The OD of the standard was also read.

$$\text{Concentration of Sample} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of Standard}$$

ERYTHROCYTE GLUTATHIONE PEROXIDASE ASSAY

Principle: Glutathione peroxidase catalyses hydrogen peroxide by the oxidation of GSH according to the reaction below:

$$2GSH + H_20_2 \xrightarrow{GPX} GSSH + H_20$$

$$GSSG + NADPH \xrightarrow{GR} 2GSH + NADP$$

Rather than measuring the progressive loss of GSH; however, this substrate is maintained at a constant concentration by the addition of exogenous GR and NADPH, which immemdiately convert any GSSG produced to GSH. The rate of GSSG formation, representing the GPX activity is then measured by following the decrease of NADPH in absorbance of the reaction mixture at 340nm.

Procedure: 50µls of RBC is mixed with 1ml of cyanodilution mixture and then shaken. 500µl of potassium was then added to 0.02ml of diluted RBC. In this assay, Cumene hydroperoxide is used as the peroxide substrate (ROOH), and Glutathione Reductase (GSSG-R) and NADPH (B- Nicotinamide Dinucleotide Phosphate) Reduced are included in the reaction mixture. The changes in A340 due to NADPH oxidation is monitored and is indicative of GPX activity. Cumene Hydroperoxide is used to measure the total GPX activity. This substrate is suitable for the reaction because it has a low spontaneous reaction with GSH low spontaneous hydrolysis and is not metabolized by catalase, one of the other universally present antioxidant enzyme.

VITAMIN E ASSAY

Procedure:1.0ml of absolute ethanol was pipette into each of two glass-stoppered test tubes. The sample tube was marked tube 1 and the blank was marked tube 2. 1.0ml of serum was added to the sample tube, the serum was added slowly with shaken to obtain a finely divided protein precipitate. 1.0ml of distilled water was added to the blank. 1.0ml of working standard and distilled water was added to a third glass-stoppered test tube marked ‘standard’.

Each of the tubes was added 1.0ml of reagent-grade xylene. The tubes were tightened and shaked vigorously for at least 30 sec. The tubes were centrifuged for 5mins at an RCF of 350-450. 0.5ml of the xylene (top) from each tube was pipetted into properly labelled 10mm round cuvettes and then 0.5ml of TPTZ solution was added and mixed. The readings were made in a spectrophotometer. It was set to zero absorbance with the blank at 460mμ and the absorbance of the sample was measured within 4 minutes. Then, 0.1ml of ferric chloride solution was added to each cuvet at definite, timed intervals and mix. Again, the instrument was set to zero absorbance with the blank and the absorbance of both the sample and the standard was measured at 600mμ. The colour continues to fade with time but fading is proportional in the sample and the standard up to 12mins. After adding the ferric chloride solution.

$$\frac{\text{Absorbance of sample}_{600m\mu} - (0.40 \times \text{absorbance of sample}_{460m\mu})}{\text{Absorbance of Standard}_{600m\mu}} = \text{Vitamin E (mg. /100ml)}$$

ERYTHROCYTE CATALASE ASSAY

Principle: The ultraviolet absorption of hydrogen peroxide can be easily measured at 240nm. On the decomposition of hydrogen peroxide with catalase, the absorption decreases with time and from this decrease, catalase activity can be measured.

Procedure: Red blood cell lysate is prepared by adding 1.2ml of distilled water to 0.02ml of RBC. Then five hundred-fold dilution of RBC lysate by phosphate buffer is made before the determination of catalase activity immediately following the addition of 1ml phosphate buffer (blank) or hydrogen peroxide solution into 2ml RBC diluted lysate. The change of absorbance of RBC against blank at 240nm is recorded every 15 seconds for 1minute on a spectrophotometer. The activity of catalase is calculated by using the following equation:

Catalytic concentration (unit/l) = (0.23log A1/A2)/0.00693

Where:

A1 =A240 at t =0

A2 =A240 at t =15 seconds

ERYTHROCYTE SUPEROXIDE DISMUTASE ASSAY:

Principle: Superoxide dismutase (SOD) reduces superoxide to hydrogen peroxide. The theory of this method is based on the competition between SOD activity and

iodonitrotetrazolium violet in reacting with superoxide, which is generated by xanthine Oxidase (XOD) reaction. The reactions are demonstrated below.

1. Xanthine + 0_2 ⟶ Uric acid + 0_2^-
2. $0_2^- + 0_2^- + 2H^+ \xrightarrow{XOD}$ Hydrogen peroxide + 0_2^-
3. 0_2^- + violet $\xrightarrow{SOD}$ Oxidized detector (generate colour)

With increasing SOD concentration, the competition between reaction 2 & 3 measured as a decrease of the rate of the detector reaction. The SOD activity measured is related to 50% inhibition of the detector reaction.

Procedure: 100µls of RBC is mixed with 900µls of distilled water. Then, 60µls of RBC lysate is further diluted with 940µl of 10mM phosphate buffer, and this diluted RBC lysate is used for the superoxide dismutase assay. 94µls of superoxide dismutase standard or diluted RBC lysate sample and 1.2ml of carbonate buffer are added into a cuvette. Just before reading the change of absorbance, 75µl of xanthine oxidase is added. Absorbance is read every 20 seconds continuously for 3 minutes on a spectrophotometer at 500nm at room temperature. The changing rate of absorbance is used to determine superoxide dismutase activity. Concentration of superoxide in samples is determined by the comparison with the calibration curve from SOD standards.

4.1 RESULTS

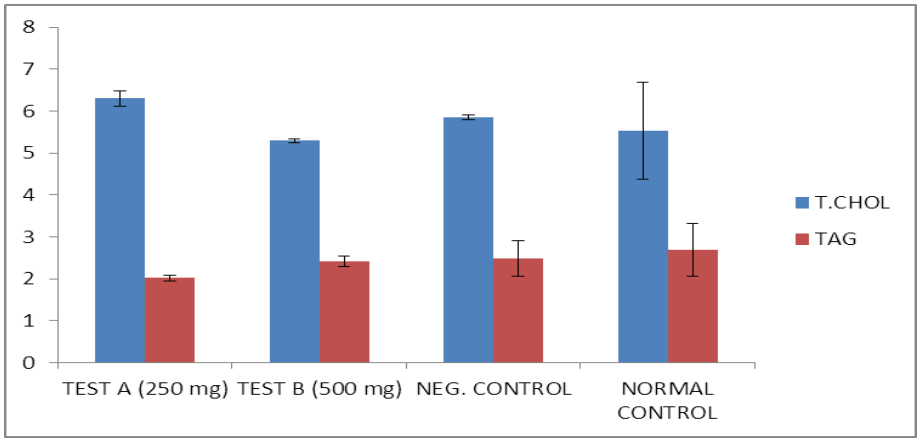

Fig. 4.1 Effect of the Methanol Extract of *Dialium Guineense* on Total Cholesterol And Triacylglycerides

From fig 4.1. above: The mean value result of the 250mg extract (6.30±0.19) and 500mg extract (5.29±0.05) on total cholesterol increased significantly ($p<0.05$) in comparison with

the Negative Control (5.86±0.06) but they have a non significant (p>0.05) increase when compared with the Normal Control (5.54±1.16).

The mean value result of the 250mg extract (2.02±0.07) and 500mg extract (2.42±0.12) on TAG had no significant (p >0.05) increase in comparison with the Negative Control (2.48±0.43) and the Normal Control (2.68±0.63).

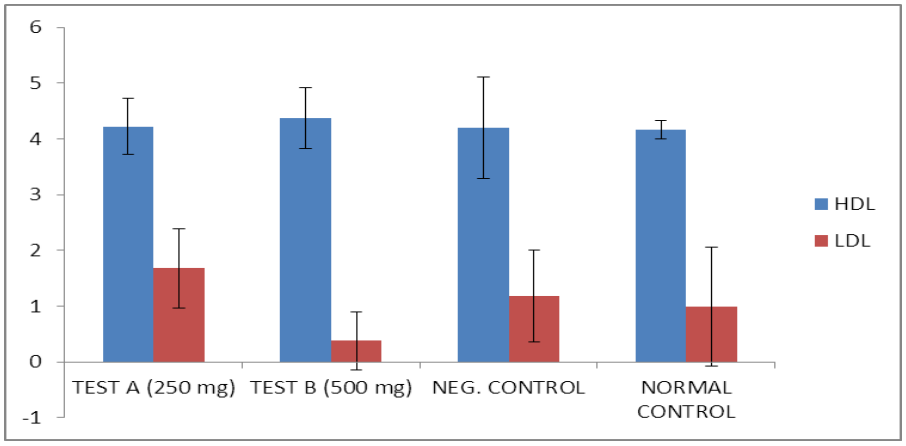

Fig 4.2: Effect of the Methanol Extract of *Dialium Guineense* on HDL and LDL

From fig 4.2, above : The mean value result of the 250mg extract (4.22±0.51) and 500mg extract (4.37±0.55) on HDL increased non significantly (p>0.05) when compared with the Negative Control (4.20±0.91) and the Normal Control (4.16±0.16).

The mean value result of the 250mg extract (1.68±0.71) and 500mg extract (0.38±0.52) on LDL increased non significantly (p>0.05) when compared with the Normal Control (0.99±1.06), but the 250mg increased significantly (p<0.05) in comparison with the Negative Control (1.18±0.82). The 500mg extract increased non significantly (P>0.05) with the Negative control (1.18±0.82)

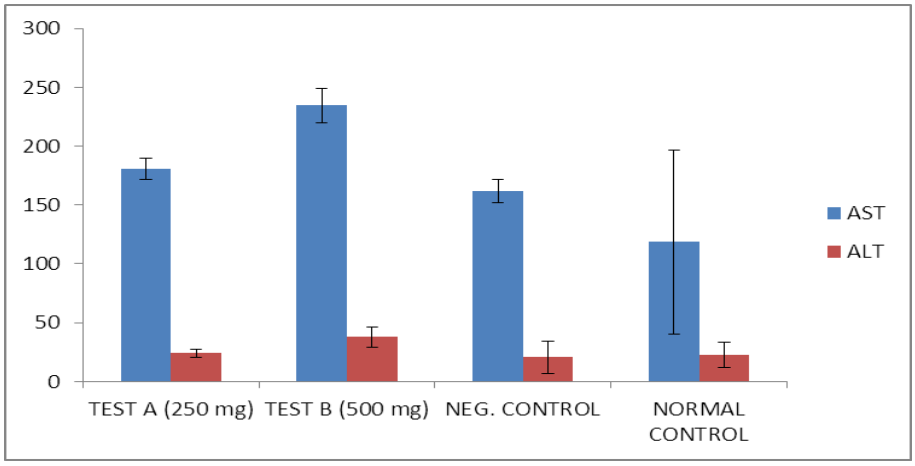

Fig. 4.3 Effect of Extract of Methanol Extract of *Dialium Guineense* on AST and ALT

From fig. 4. 3 above: The mean value result of the 250mg extract (180.58±9.29) and 500mg extract (234.43±14.70) on AST increased non significantly (p>0.05) and significantly (P<0.05) respectively when compared with the Negative Control (161.73±10.22) and increased non significantly (p>0.05) in comparison with the Normal Control (118.57±78.16). The mean value result of the 250mg extract (24.10±3,29) and 500mg extract (37.98±8.57) on ALT increased non significantly (p>0.05) significantly (p<0.05) respectively when compared with the Negative Control (20.53±13.93) but they inreased non significantly (p>0.05) in comparison with the Normal Control (22.68±10.69).

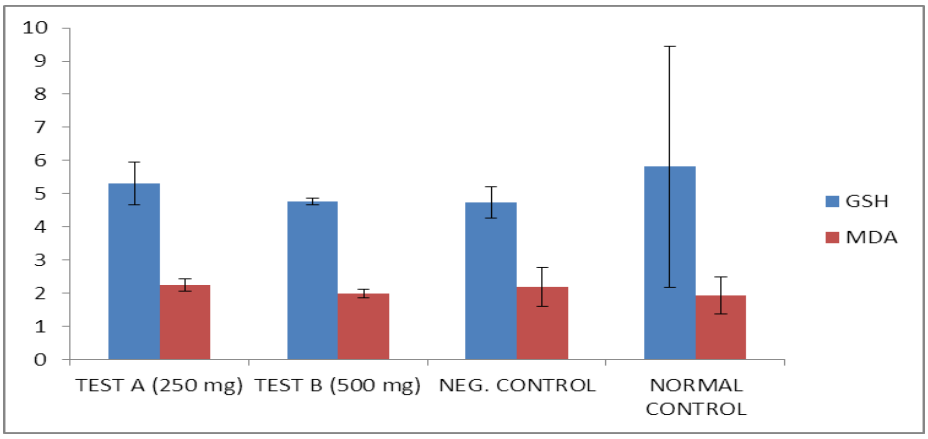

Fig 4.4 Effect of the Methanol Extract of *Dialium Guineense* on GSH and MDA

From fig.4.4 above: The mean value result of the 250mg extract (5.30±0.64) and 500mg extract (4.77±0.10) on GSH increased non significantly (p>0.05) when compared with the Negative Control (4.73±0.48) and the Normal Control (5.80±3.64).

The mean value result of the 250mg extract (2.25±0.19) and 500mg extract (1.98±0.13) on MDA increased non significantly (p>0.05) when compared with the negative Control (2.18±0.59) and the Normal Control (1.93±0.55).

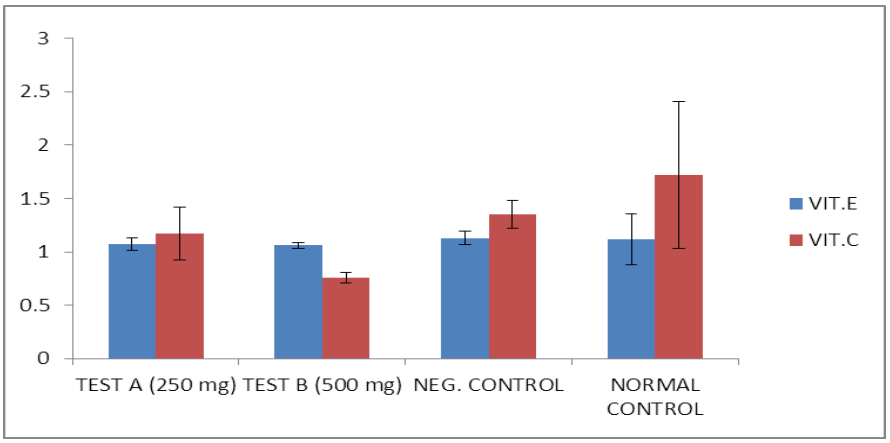

Fig. 4.5 Effect of the Extract on Vitamin E and Vitamin C

From fig.4.5 above: The mean value result of the 250mg extract (1.07±0.06) and 500mg extract (1.06±0.03) on VIT. E had a significant ($p<0.05$) increase when compared with the Negative Control (1.13±0.06) but a non significant ($p>0.05$) increase in comparison with the Normal Control (1.12±0.24).

The mean value result of the 250mg extract (1.17±0.25) and 500mg extract (0.76±0.05) on VIT. C had a non significant ($p>0.05$) increase and a significant ($p<0.05$) increase respectively when compared with the Negative Control (1.35±0.13) but they had a non significant ($p>0.05$) in comparison with the Normal Control (1.72±0.69).

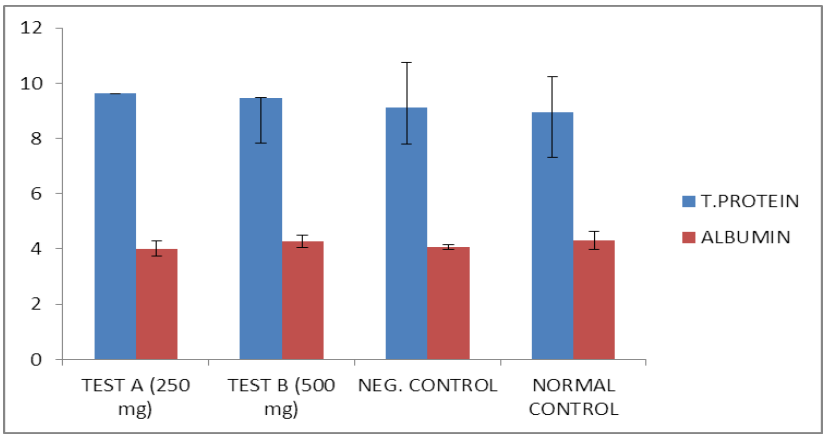

Fig. 4.6 Effect of the Extract on T.Protein and Albumin

From fig. 4.6 above: The mean value result of the 250mg extract (9.63±0.02) and 500mg extract (9.45±1.63) on T. PROTEIN had a non significant ($p>0.05$) increase when compared with the Negative Control (9.11±1.31) and Normal Control (8.93±1.6).

The mean value result of the 250mg extract (4.01±0.28) and 500mg extract (4.27±0.23) on ALB. had a non significant increase ($p>0.05$) when compared with the Negative Control (4.08±0.09) and the Normal Control (4.29±0.33).

From fig.4.7 above: The mean value result of the 250mg extract (1.07±0.57) and 500mg extract (0.99±0.01) on SOD. had a significant ($p<0.05$) increase when compared with the Negative Control (0.91±0.05) but had a non significant ($p>0.05$) increase when compared with the Normal Control (0.99±0.11).

The mean value result of the 250mg extract (1.22±0.04) and 500mg extract (1.19±0.05) on CATALASE had a significant ($p<0.05$) increase when compared with the negative Control

(3.09±0.61) and a non significant (p>0.05) increase in comparison with the Normal Control (2.95±2.11).

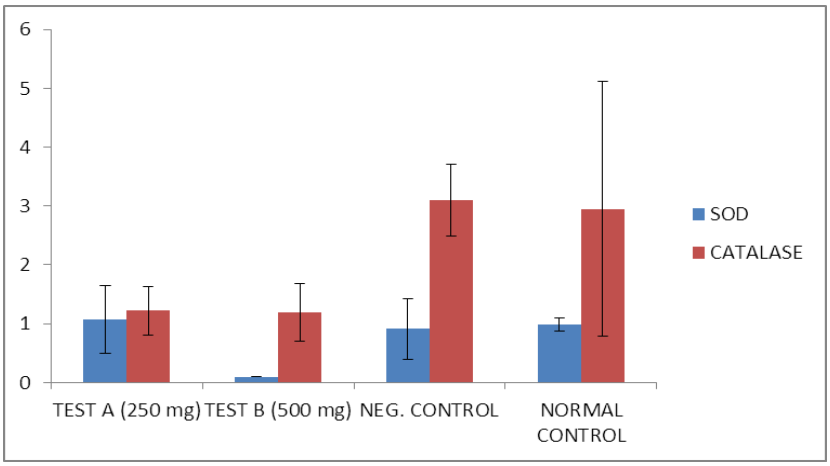

Fig. 4.7 Effect of the Extract on Superoxide Dismutase and Catalase.

DISCUSSION

As a result of considerable attention that has been devoted to the liver diseases, the vast majority of studies have been concerned with the liver of the experimental animals and the characteristics of animal model's normal liver have been well defined.

Chloroform/hexane is one of the hepatotoxins that cause liver damage. The hepatotoxicity of chloroform was reported to be due to phosphogene-mediated cellular glutathione depletion or increased amounts of covalent binding to hepatocellular macromolecules (Purushothum et al., 1998; Burke et al., 2007).

ALT and AST are important biochemical markers of hepatotoxicity in blood plasma and serum. ALT is a liver enzyme that aids in amino acid metabolism and gluconeogenesis, catalyzing the reductive transfer of an amino group from alaine to α-ketoglutarate to yield glutamate and pyruvate, AST aids in producing proteins, catalyzing the reductive transfer of an amino group from aspartate to α-ketoglutarate yielding oxaloacetate and glutamate.

In this present study, it is observed that the test group treated with the 250mg extract of *Dialium guineese* had a non-significant (P>0.05) increase on AST activity when compared with the negative control and normal control. The 500mg extract on AST activity had a significant (P<0.05) increase when compared with the negative control but increased non significantly (P>0.05) in comparison with the normal control. This means that the extract has no effect in reversing the damage done by the hepatotoxicant and as such can contribute to

liver damage. Also, the 250mg extract had an increase in ALT activity but in a non significant (P>0.05) manner when compared with the negative and normal control. The 500mg extract fed to the test groups had a significant (P<0.05) increase on ALT activity when compared with negative control but increased non significantly (P>0.05) in comparison with the normal. Therefore, the extract could even help in damaging the hepatocyte.

Lipids are of important functions. A lipid profile is a measurement of various lipids that normally circulate in the blood. Cholesterol is a precursor to many important biological hormones such as oestrogen and testosterone. LDL is referred to as the "bad cholesterol" lipoprotein. They carry cholesterol from the liver to cells of the body. HDL collect cholesterol from the body's tissues, and bring it back to the liver and are known as the 'good cholesterol" lipoprotein. Triglycerides is the most common type of lipid formed in animals. Fat tissue is primarily for the storage of this form of lipid. From the result of this study, 250mg and 500mg extract of *Dialium guineense* had a non significant (P>0.05) increase on TAG when compared with the negative and the normal control whereas the 250mg and 500mg extract on total cholersterol increased significantly (P<0.05) when compared with the negative control, but a non significant (p>0.05) increase in comparison with the normal control. 250mg and 500mg extract on HDL increased non significantly (P>0.05) when compared with the negative and the normal control. There was no significant (p>0.05) increase in 250mg and 500mg extract on LDL against the normal control. But the 250mg increased significantly (P<0.05) against the negative group, while the 500mg increased non significantly (p>0.05) against the negative control.

Malondialdehye (MDA) is formed by radical-mediated lipid peroxidation and shows mutagenic and cytotoxic effects by further reaction with DNA. MDA is the end product of lipid peroxidation and a good marker of free radical-mediated damage and oxidative stress (Atip *et al.*, 2010). In this result, neither the 250mg extract nor 500mg extract had a significant (P<0.05) increase when compared with negative and normal group.

Antioxidants are substances that slow oxidative stress and lipid peroxidaton damage to cellular components such as DNA, proteins and lipid. They are also molecules which safely interact with free radicals and terminate free radical chain before they cause damage to cells (Njoku et al., 2011). In this result,250mg and 500mg 0n vitamin E and Catalase had a significant (p<0.05) increase when compared with the negative control. 500mg extract on Vitamin C and Superoxide Dismutase had a significant (p<0.05) when compared with the

negative control. This shows that the extract is a good free radical scavenger. Others do not have a significant increase against the negative and the normal control.

Total protein is important because its estimation is helpful in differentiatng between a normal and a damaged liver function as the majority of plasma proteins like Albumins and globulins are produced in the liver (Thepa and Walia, 2007). From this result, the 250mg and 500mg extract had a non significant($p>0.05$) increase on albumin against the negative control and the normal group. Also, 250mg and 500mg extract on total protein had a non significant ($p>0.05$) increase in comparison with the negative and normal control.

CONCLUSION

The hepatoprotective screening of Dialium Guineense reveals that it has no protective effect on ALT and AST which are the important biochemical markers of hepatotoxicity. This is because it caused an increase in the levels of AST and ALT in a dose dependent of 500mg. Both doses exert an increase effect on total cholesterol and HDL except HDL which increased on a 250mg dose. Interestingly, both the 250mg and the 500mg extract exert an increase effect on vitamin C and E as well as catalase and superoxide Dismutase. Therefore, *dialium guineense* has no effect on the liver cell damage and lipid profile but it is a good source of antioxidant and should be recommended.

REFERENCES

1. Bleibel W., Kim S., D'Silva K., Lemmer ER. (2007). Drug-induced liver injury: Review Article. Dig Dis Science, 2007; 52: 2463-2471.
2. Burke AS., Redeker K., Kurten, RC., James LP., Hinson JA. (2007) Mechanisms of chloroform-induced hepatotoxicity: Oxidative stress and mitochondrial permeability transition in freshly isolated mouse hepatocytes. Journal Toxicology Environ Health, 2007; 20: 1936-1945.
3. Chang F.Y., Lee S.D. (2003). Cytochrome P450 2E1genotype and the susceptibility to antituberculosis drug-induced hepatitis. Hepatology. 2003; 37: 924–930.
4. Deng X., Luyendyk JP., Ganey PE., Roth RA. (2009). Inflammatory stress and idiosyncratic hepatotoxicity: Hints from animal models. Pharmacology Rev, 2009; 61: 262-282.
5. Grant G.H. (1987). Amino acids and proteins, fundamentals of Clinical chemistry, Tietz N, W, Editor. Third Edition, WB Saunders company Philadelphia USA, 1987; 328-329.

6. Naruse K., Tang W., Makuuchi M. (2007). Artificial and bioartificial liver support: A review of perfusion treatment for hepatic failure patients. World Jourrnal Gastroenterology, 2007; 13: 1516-1521.
7. Njoku D., Laster M.J. (1997). Gong D.H. Biotransformation of halothane, enflurane, isoflurane and desflurane to trifluoroacetylated liver proteins: association between protein acylation and hepatic injury. Anest Analg. 1997; 84: 173-178.
8. Ostapowicz G., Fontana R.J., Schiodt F.V., Larson A., Davron J.T., Steven H.B., Timothy M., Reish J. (2002). Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann International Med. 2002; 137: 947–954.
9. Purushothum KR., Lockard VG., Mehendale HM. (1998) Amplification of chloroform hepatotoxicity and lethality by dietary chlordecone (Kepone) in mice. Toxicology, Pathology, 1998; 16: 27-34.
10. Reitman, S., Frankel, S. (1957). American Journal. Clinical Pathology. 1957; 28:56.
11. Saukkonen JJ., Cohn DL., Jasmer RM., Schenker S., Jereb JA. (2006). An Official ATS Statement: Hepatotoxicity of antituberculosis therapy. Am J Respir Crit Care Med, 2006; 174: 935-952.
12. Sharma A., Chakraborti K.K., Handa S.S. (1991). Anti-hepatotoxic activity of some Indian herbal formulations as compared to silymarin. Fitoterapia. 1991; 62: 229-235.
13. Subramonium A., Pushpangadan P. (1991) Development of Phytomedicines for liver diseases. Indian Journal Pharmacology, 1991; 31: 166-175-50.
14. Thapa BR, Walia A. (2007) Liver function tests and their interpretation. Indian Journal Pediatrics. 2007; 74: 663-671.