EFFECTS OF METHANOL EXTRACT OF *ACIOA BARTERI* ON HEPATOCellular DAMAGE AND LIPID PROFILE OF ALBINO RAT

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ABSTRACT

Methanol extract of *Acioa barteri* was evaluated for hepatoprotection and lipid profile activities in rats. The plant extract at (250 and 500mg/kg) showed a significant (P<0.05) hepatoprotective activity against chloroform and hexane induced hepatotoxicity as judged from the serum marker Enzymes and antioxidant levels in liver tissues. Chloroform and hexane induced a significant rise in Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Lipid Peroxidase (LPO), Triglyceride and total cholesterol with a reduction of total protein, Superoxide Dismutase (SOD), Catalase, Glutathione Peroxidase, VIT C and VIT E. Treatment of rats with different doses of plant extract (250 and 500mg/kg) significantly altered serum marker Enzymes (AST and ALT), antioxidant (MDA, GSH, CAT and VIT C) and Lipid profile (TAG) levels to near normal against the chloroform and Hexane induced treated rat, when compared with the controls.

KEYWORDS: Methanol extract, *Acioa barteri*, hepatoprotection.
INTRODUCTION
Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. The study of traditional medicine has evolved over the millennia of human existence. Nature has been a source of medicinal agents for thousands of years and impressive number of modern drugs has been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine.

Medicinal plants are local heritage of global importance and continue to play an essential role in health care with 80% of the world’s inhabitants relying mainly on traditional medicines for their primary health care (Cragg et al., 2001). An account of 70% of the India, 80% of Pakistan and 80% of Nepal are dependent on traditional plant-based medicines (Kumer and Peterman, 2013) medicinal plants help in alleviating human suffering. Ethnomedicinal studies are a suitable source of information regarding useful medicinal plants that can be targeted for domestication and management. The components of ethnomedicine have long been ignored by many biomedical practitioners for various reasons, for example, the chemical composition, dosages and toxicity of the plants used in ethnomedicine are not clearly defined. However, it is interesting to note that the ethnomedicinal uses of plants is one of the most successful criteria used by the pharmaceutical industry in finding new therapeutic agents for the various fields of biomedicine. Today most plant parts such as root, stem, leaves, flowers, fruits, seeds, and so on are used in various forms of medicine.

Clearly, the identification of plant materials that can manage liver disease and its complications would save millions of people especially in developing countries. The liver is the key organ regulating homeostasis in the human body. The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. (Ferguson and Harris, 1993). Jaundice and hepatitis are two major hepatic disorder that account for a high death rate. (Wild et al, 2004). Presently, a few hepatoprotective drugs formed from natural sources are available for the treatment of liver disorder. Hence, people are looking at the traditional system of medicine for remedies to hepatic disorders.

BACKGROUND OF STUDY
The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and production (Bandito et al., 1987). The liver is expects not only to perform
physiological function but also to protect against the hazards of harmful stags and chemicals. Inspite of tremendous scientific advancement in the field of hepatology in recent years, liver problem are on the rise. (Bronson et al., 1996).

Liver function test can be classified into five classes according to the function of the liver as given below.

**Test based on excretory function**
An important physiologies role of the liver is the removal of potentially noxious endogenous and Exogenous substances from the blood and there after excretion into the bile or conversion to products suitable for excretion by the kidney or lungs.
This test includes measurement of
- Serum bilirubin
- Urine bilirubin
- Urine and faecal urobilinogen
- Urine bile salts
- Dye excretion test
- Indo cyanine green (ICG) or cyanine
- Bromosulphthalein (BSP) dye tests

**Tests based on detoxification function**
The liver is involved in the detoxifications and removal of potentially hazardous substances from the body. These may be endogenous or exogenous. (Faucett and Scott, 1996) this test includes determination of
- Blood ammonia
- Bilirubin
- Hippuric acid test

**Tests based on synthetic function**
The liver is responsible for a variety of synthetic activities. It includes determination of
- Plasma proteins,
- Albumins
- Globulins
- Prothrombin time.

**Test based on metabolic function**
A number of hepatic function tests have been based on the role of the liver in intermediary metabolism. It includes

Test related to carbohydrate metabolism such as galactose intolerance tests. Test related to lipid metabolism such as determination of serum cholesterol and ratio of free to esterified cholesterol. Test related protein estimation, serum ammonia estimation, amino acid in urine.

**Determination of serum enzymes**

In liver cell injury, damage to the membrane cells and organelles allows intercellular enzymes to leak into the blood, where their elevated concentration can be measured. (Bartel and Botimer, 1972) A large numbers of different enzymes have been used in the diagnosis of liver disease. But most commonly and routinely employed in laboratory are

- serum Alanine Aminotransferase (ALT)
- serum Aspartate Aminotransferase (AST)
- serum Alkaline Phosphatase (ALP)

Other enzymes which have been found to be useful but not routinely done in the laboratory are

- Serum -5- nucleotidase
- Lactate dehydrogenase
- isocitrate dehydrogenase
- glutamyl transferase.

**OBJECTIVE OF STUDY**

This work is aimed at evaluating the effectiveness of the methanol extract of *Acioa bateri* in the estimation of hepatoprotective potentials and lipid profile.

**Table 1: Some plants having hepatoprotective potentials**

Source: http://www.newscientists.com

**MATERIALS AND METHODS**

During the course of this study, a number of equipment and reagents were used. These include beakers (Medical Expo), test tubes (Medical Expo), anticoagulant bottles (EDTA bottles), plain specimen bottles, capillary tubes, PCV hematocrit reader, PCV hematocrit centrifuge, filter papers (Whitman no. 1), electronic weighing balance (Sartorius sensitive
weighing balance-1200g), pipettes, micropipettes, measuring cylinders (Pyrex, England), water bath, light (binocular) microscope (Olympus), Spectrophotometer (UV-Visible spectrophotometer – UK, Unicom Ltd), centrifuge, oven (Tenor and Empress Registered ER109/1/96180 9001 Italy), refrigerator, conical flasks, metabolic cages, gavage and insulin syringes, reagent bottles (Medical Expo), dissecting set, Succinate buffer, bromocresol green, Phosphate buffer, Sulphamilic acid, Sodium nitrate, Caffeine, Sodiumhydroxide, Pine acid.

PREPARATION OF PLANT EXTRACT

Fresh leaves of the plant *Aciola Barteri* were obtained from a local farm in Umuaria village, Umudike, Abia State, Nigeria, and identified by Dr. Garuba Omosun of the Plant Science and Biotechnology department, Michael Okpara University of Agriculture, Umudike. The leaves (100g) of the plant collected were washed and dried and then blended into powder.

About 100g of the powdered leaves of *Aciola Barteri* soaked in methanol for two days, after which the extract was filtered using a Whatman no. 1 filter paper and then the filtrate was allowed to evaporate to dryness and then used for the study.

EXPERIMENTAL ANIMALS

Healthy female Wistar albino rats having 130 grams mean weight were used for the study. All animals were housed in an animal house under normal room conditions and acclimatized for two (2) weeks. Commercial pellet diet (Vital growers mash by Grand Cereals and Oil Mills, Nigeria) and water were given to the animals ad libitum.

INDUCTION OF HEPATOTOXICITY

All the rats used for this study were initially subjected to hepatocyte damage using hexane and chloroform in the ratio of 4:6 at 2.5ml per rat orally except the normal control group.

EXPERIMENTAL DESIGN

Sixteen female albino Wistar rats were used for the study. They were acclimatized in the animal house for two weeks before beginning the experiment. The animal were randomized according to their weights and housed in four stainless steel cages containing four rats each: gp1, gp2, gp3 and gp4. The first two being the test groups and the last two the control groups. Test Groups were given 250, 500mg/kg body weight respectively of methanolic leaf extract of *A. barteri*, 10 days via gastric incubation. Group three represented the normal control that did not receive the extract, but food and water only. Group four represented the negative
control to which hepatocyte damage was induced with chloroform and hexane and treated with neither the extract nor standard drug.

**ASSESSMENT OF LIVER FUNCTION**

Biochemical parameters, that is, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein, total cholesterol, triglycerides, HDL and LDL, analyzed according to the reported methods. The liver was removed, morphological changes were observed. A 10% of liver homogenate was used for antioxidant studies such as lipid peroxidation, superoxide dismutase, catalase, glutathione, VIT E and VIT C.

**Determination of hepatocellular parameters**

**Aspartate Aminotransferase (AST)**

**Principle and Procedure**

\[
\alpha - \text{oxoglutarate} + L - \text{aspartate} \xrightarrow{GOT} L - \text{glutamate} + \text{ovaloacetate}
\]

AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4 dinitrophenylhydrazine. Method for AST (measurement against reagent blank) 0.1ml of the serum was added to the 0.5ml initial concentrations of solutions in the test tube and about 0.1ml of distilled H\textsubscript{2}O was pipette into it and was mixed vigorously and incubated for exactly 30mins at 37\textdegree C.

The 0.5ml of 2, 4 dinitrophenylhydrazine was mixed to the solution and was allowed to stand for exactly 20mins at 25\textdegree C. 5.0ml of sodium hydroxide was mixed with the present concentration; the absorbance of sample was read against the reagent blank after 5mins. The experiment was carried out again for measurement against sample blank (Schmidt and Schmidt 1963)

**Alanine Aminotransferase (ALT)**

**Principle and Procedure**

\[
\alpha - \text{oxoglutarate} - L - \text{alanine} \xrightarrow{ALT} L - \text{glutamate} + \text{pyruvate}
\]

Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4 diniitrophenylhydrazine.

0.1ml of the serum, 0.5ml of the initial concentration of the serum and 0.1ml of distilled H\textsubscript{2}O was mixed together in a test tube and incubated for exactly 30mins at 37\textdegree C. After incubation, 0.5ml of 2, 4 dinitrophenylhydrazine was mixed together with the solution and allowed to
stand for exactly 20min at 25°C. 5.0ml of sodium hydroxide was mixed with the present concentration the absorbance of the sample was read against the reagent blank after 5minutes. The experiment was repeated for measurement against sample blank (Schmidt and Schmidt 1963).

**Total Protein**

**Principle and Procedure**
At total alkaline ph value, protein forms a stable complex with copper II ions, which is photometrically measured.

The procedure used was according to the method of Peter and Wiechselbaum (1968).

**Albumin (ALB)**

**Principle and Procedure**
The measurement of serum albumin is based on its quantitative binding to the indicator 3, 3’, 5, 5’, tetrabromocresol sulphonephthalein (bromocresol green, BCG). The albumin – BCG – complex absorb maximally at 578nm, the absorbance being directly proportional to the concentration of albumin in the sample.

The procedure used was the method, according to Grant et al 1987.

**Estimation of antioxidant activity**

**Malondialdehyde (MDA) (Lipid peroxidation)**

**Principle and Procedure**
Malondialdehyde (MDA) reacts with thiobarbituric acid to form a red or pink coloured complex which in acid solution, absorbs maximally at 532nm.

\[
\text{MDA} + 2\text{TBA} \rightarrow \text{MDA: TBA adduct} + \text{H}_2\text{O}
\]

0.1ml of the sample + 0.9ml of distilled H2O + 0.5ml of 25% TBA + 0.5ml of 1% TBA were added to 0.3% NaOH in a test tube. This conc. was incubated at 95°C for 40mins after the incubation, 1ml of 20% SDS (sodium dodosyl sulphate was then added to the solution. The absorbance was determined at 532 and 600nm against a blank (Rosengren et al., 1993)

Thus;

\[
\% + \text{BARS} = \frac{A_{532} - A_{600}}{0.5271 \times 0.1} \times \frac{100}{1} = 0.52080 \text{mg/ml}
\]
Glutathione (GSH)

Principle and Procedure
Glutathione peroxidase catalyses hydrogen peroxide by the oxidation of GSH according to the following reaction:

\[
2\text{GSH} + \text{H}_2\text{O}_2 \xrightarrow{\text{GPX}} \text{GSSG} + \text{H}_2\text{O} \\
\text{GSSG} + \text{NADPH} \xrightarrow{\text{GR}} 2\text{GSH} + \text{NAD}
\]

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

50µl of RBC is mixed with 1ml Cyanodilution mixture and then shaken. 500ul of potassium was then added to 0.02ml of diluted RBC (Achi 1983).

Tocopherol (VIT E)

Principle and Procedure
Vit E acts as a natural antioxidant by scavenging from radicals and molecular oxygen. It is important for preventing peroxidation of polyunsaturated fatty acids in CCU membranes. Vitamin E terminates free radical lipid peroxidation by donating single electron to form the stable fully oxidized tocopherol quinine. Protection of erythrocyte membrane from oxidant is the major role of vitamin E in humans. (Pankaja, 2005).

0.1ml of the serum was pipette into a test tube and 0.9ml of distilled H$_2$O was added. 1ml of 0.2% ferric chloride and 1ml of alcoholic 0.5% dipyridyl solution and shaked after which 5ml was diluted with distilled H$_2$O and the absorbance measured at 520nm (Harmon et al., 1991).

Catalase

Principle and Procedure
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.
Red blood cell lysate is prepared by adding 1.2ml of distilled H\textsubscript{2}O to 0.2ml of RBC. Then five hundred fold dilution of RBC lysate by phosphate buffer was made before the determination of catalase activity.

Immediately after the addition of 1ml of hydroxide peroxide solution into 2ml RBC diluted lysate, the change of absorbance of RBC against blank at 240nm was recorded at 15 seconds interval for 1 minute on the spectrophotometer (Achi 1983).

**Ascorbic acid (VIT C)**

**Principle and Procedure**

Ascorbic acid is oxidized and converted diketoglutamic acid in strong acid solution and plasma separated from the blood cells. These plasma samples were analysed as soon as possible.

10g of EDTA was dissolved in distilled H\textsubscript{2}O and the volume of measured and about 0.05ml of EDTA was added to the solution. It was then allowed to evaporate to dry salt at room temperature before they were used (Rosengren et al, 1993).

**Superoxide Dismutase (SOD)**

**Principle and Procedure**

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 reading with superoxide, which is generated by xanthine oxidase (XOD) reaction. The reactions are demonstrated below;

\[\text{a. } \text{Xanthine} + \text{O}_2 \rightarrow \text{Uric acid} + \text{O}_2\]
\[\text{b. } \text{O}_2 + \text{O}_2 + 2\text{H} \xrightleftharpoons{}^{\text{XOD}} \text{hydrogen peroxide} + \text{O}_2\]
\[\text{c. } \text{O}_2 + \text{violet} \xrightarrow{}^{\text{SOD}} \text{oxidized detector (generate color)}\]

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

100\text{µl} of RBC was mixed with 900\text{µl} of distilled water and then 60\text{µl} of RBC lysate was further diluted with 940\text{µl} of 10mm phosphate buffer and this diluted RBC lysate was used for the superoxide dismutase assay. 94\text{µl} of superoxide dismutase standard of diluted RBC lysate sample and 1ml of carbonate buffer was added into a cuvette. After which 75\text{µl} of
xanthine oxidase was added and the reading of the absorbance was recorded (Waterman et al., 1991).

**Determination of Lipid profile**

**Total cholesterol**

**Principle and Procedure**
The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-amino antipyrine in the presence of phenol and peroxidase. This method was carried out according to procedure of Richmond and Clinchem(1973).

**HDL AND LDL**

**Principle and Procedure**
Iow density lipoprotein and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ion. After centrifugation, the chloeresterol concentration in the HDL fraction which remains in the supernatant is determined.

This procedure was carried out according to the method provided by Abell et al. (1992).

**Triglycerides**

**Principles and Procedure**

\[ \text{Triglyceride} + \text{H}_2\text{O} \xrightarrow{\text{lipases}} \text{glycerol} + \text{fatty acids} \]

\[ \text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol} – 3-\text{phosphate} + \text{ADP} \]

\[ \text{Glycerol} – 3-\text{phosphate} + \text{O}_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone} + \text{phosphate} + \text{H}_2\text{O}_2 \]

\[ 2\text{H}_2\text{O}_2 + 4 \text{aminophenazone} + 4 \text{chlorophenol} \xrightarrow{\text{POD}} \text{quinoneimine} + \text{HCL} + 4\text{H}_2\text{O} \]

This procedure was carried out according to the method provided by Koditscheck et al. (1969).

**Statistical Analysis**
The data are expressed as mean ± Standard deviation. Comparison between animals administered with the extract consisting of two groups treated with different doses of the extract were performed using one way annova test. Significance was accepted at (P<0.05).
RESULT AND INTERPRETATION

4.1.1 Effect of Methanolic Extract of *Acioa Barteri* on Hepatocellular Parameter

![Figure 1: Effect of Methanolic Extract of *Acioa Barteri* Hepatocellular Parameter](image)

The result above shows a significance (p<0.05) decrease when the AST and ALT levels are compared between the 500mg extract and control respectively. But, total protein and Albumin shows a non significant (p>0.05) when compared with the control.

4.1.2 Effect of methanolic extract of *Acioa barteri* on antioxidant parameter

![Figure 2: Effect of methanolic extract of *Acioa barteri* on antioxidant parameter](image)

The result above shows a significance (P<0.05) decrease when the MDA, CAT, VIT C, and GSH level compared between the independent dose of the extract (250 and 500mg/kg) and control respectively. But, VIT E and SOD shows a non-significance (P>0.05) when compared with the controls.
4.1.3 Effect of methanolic extract of *Acioa barteri* on Lipid parameter

The result above shows a significance (P<0.05) decrease when the TAG level is compared between 500mg extract and controls respectively. But, T.CHOL, HDL and LDL show a non-significance (P>0.05), when compared with the control.

**DISCUSSION**

Chloroform and hexane is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of chloroform and hexane are largely due to its active metabolite, trichloromethyl radical. (Abell *et al.*, 1992). These activated radical bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. This lipid peroxidative degradation of biomembrane is one of the principle causes of hepatotoxicity of chloroform and hexane. This is evidenced by an elevation in the serum marker enzymes namely AST, ALT, ALP and decrease in protein.

In the assessment of liver damage by chloroform and hexane, the determination of enzyme levels such as AST and ALT are largely used. Membrane damage releases the enzymes into circulation and hence it can be measured in the serum. High levels of AST and ALT indicates liver damage, such as that caused by viral hepatits as well as cardiac infarction and muscle injury, ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and
loss of functional integrity of cell membrane in liver. Serum ALP, albumin and total protein levels on the other hand are related to the function of hepatic cell.

Administration of chloroform and hexane caused a significant (P<0.05) elevation of enzymes levels such as (AST and ALT), MDA, triglycerides, total cholesterol and decrease in total protein when compared to control however there was a significant (P<0.05) restoration of AST and ALT level on administration of the leaf extract in a 500mg dose dependent manner when compare to the control. The reversal of the increased serum enzymes in chloroform and hexane induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases is normal with the healing of hepatic parenchyma and regeneration of hepatocyte. Effectively control of ALP, albumin and total protein levels points towards an early improvement is the secondary mechanism of the hepatic cells.

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin, the plant extract decreased chloroform and hexane induced elevated enzymes levels in some tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damage liver cells.

Triglycerides are stores of fatty acid with glycerol. These are composed of three fatty acid which are esterified through their carboxyl groups resulting to loss of negative charge and formation of neutral fat. Elevated concentrations are often found in disturbance of lipid metabolism and in atherosclerosis. Decreased triglyceride level is a sensitive index in the hepatoprotection or regeneration of liver damage. Treatment with the plant extract A. barteri significantly decreased the level of triglycerides.

The increase in lipid peroxidation (MDA) level in liver induced by chloroform and hexane suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radical. Treatment with A. barteri significantly reverses these changes. Hence it is likely to be that the mechanism of hepatoprotection of A. Barteri is due to its antioxidant effect.
Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissue, and the higher activity is found in the red blood cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals. Therefore the reduction in the activity of catalase may result in a number of deleterious effects due to assimilation of superoxide radical and hydrogen peroxide. A higher dose (500mg/kg) of *A. barteri* significantly increases the level of CAT.

Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. It removes free radicals species such as hydrogen peroxide, super oxide radicals and maintains membrane protein thiols. Also it is a substrate for glutathione peroxidise. Decreased level of GSH is associated with an enhanced lipid peroxidation in chloroform and hexane induced, treated rat. Treatment with *A. barteri* significantly increased the level of glutathione peroxidise in a dose dependent manner.

Ascorbic acid reduces oxidized tocopherol produced in the process of free radical scavenging by VITE and so regenerates functional tocopherol. High level of ascorbic acid is involved in the prevention of liver damage and coronary heart disease by preventing oxidation of LDL. Treatment with *A. barteri* significantly increased the ascorbic level.

**CONCLUSION**

Conclusively, considering the study, it may be stated that the methanolic extract of *A. Barteri*, will provide a new therapeutic avenue against hepatocyte damage and liver related complications. Moreover, further works is necessary to search out the active ingredients present in this extract having hepatoprotective potential. Therefore, based on the results of this study, the leave of *A. Barteri* should be screened in various forms and encouraged for consumption based on it rich nutritional profile and hepatoprotective potentials.

**REFERENCES**


