EFFECT OF METHANOL EXTRACT OF GAMBIA ALBIDUM LEAVES ON CCL₄-INDUCED HEPATOCYTLULAR DAMAGE AND LIPID PROFILE IN ALBINO RATS

Aloh, G.S.¹, *Obeagu, Emmanuel Ifeanyi², Odo Christian Emeka after¹, Okpara, Kingsley Ezechukwu³ and Ugwu Getrude Uzoma⁴

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

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

³Rivers State College of Health Technology, Port Harcourt.

⁴School of Nursing Science, ESUT Teaching Hospital, Parklane, Enugu, Nigeria.

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ABSTRACT

This study was designed to evaluate the hepatoprotective/antioxidant properties and lipid profiles of rats given methanol leaf extract of *Gambia albidum* in hexane/chloroform (4:6)-induced hepatotoxicity. The parameters assessed were the serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione (reduced, GSH), aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), total protein, albumin, vitamins C, and E and lipoprotein fractions: HDL, LDL, triacylglycerol and total cholesterol (T.chol) levels in the positive control, negative control and the experimental rats after 10 days of extract administration. The 16 rats were divided into four groups of 4 rats per group (A-D): Groups A and B rats were given 250mg and 500mg of the extract per kg body weight respectively once daily for 10 days after inducing hepatotoxicity. Group C rats were given 2.0 ml of C₆H₁₄/CHCl₃ without any treatment, which caused a significant elevation (p<0.05) in the levels of MDA, AST and ALT, indicators of lipid peroxidation and hepatocellular damage. Group D rats received neither the hepatotoxicant nor any treatment.
but given feed and water only. After 10 days of extract administration, the Group A rats (given 250mg/kgbw) showed significant (p<0.05) increase in MDA and AST and decrease in TAG and CAT levels compared to the positive control (Group D) while T.chol and GSH increased significantly (p<0.05) compared to the negative control (Group C). The Group B rats (given 500mg/kgbw) showed significant (p<0.05) increase in AST compared to the positive control and a significant (p<0.05) decrease in GSH and CAT compared to the negative control, and TAG compared to the positive control. HDL and VIT C in Groups A and B showed a dose-dependent increase compared to Group C but non-significant (p>0.05). Also Groups A and B had a dose-dependent decrease in ALT level close to Group D level. Other parameters tested did not show any significant difference. The results of this study indicate that methanol extract of *G. albidum* exerted mild hepatoprotection and antilipidemic properties.

**KEYWORDS:** Hepatoprotective/antioxidant, *Gambia albidum*, antilipidemic properties.

**INTRODUCTION**

Reactive oxygen species (ROS) is a collective term used for a group of oxidants, which are either free radicals or molecular species capable of generating free radicals. Intracellular generation of ROS mainly comprises superoxide (O$_2^-$) radicals and nitric oxide (NO$^-$) radicals. Under normal physiologic conditions, nearly 2% of the oxygen consumed by the body is converted into O$_2^-$ through mitochondrial respiration, phagocytosis, etc (Winterbourn, 2008). Reactive oxygen species percentage increases during infections, exercise, exposure to pollutants, UV light, ionizing radiation, etc (Amit and Priyadarsini, 2011). NO$^-$ and O$_2^-$ radicals, are converted to powerful oxidizing radicals like hydroxyl radical (·OH), alkoxy radicals (RO$^-$), peroxyl radicals (ROO$^-$), singlet oxygen (‘O$_2$) by complex transformation reactions. Some of the radical species are converted to molecular oxidants like hydrogen peroxide (H$_2$O$_2$), peroxynitrite (ONOONO$^-$), hypochlorous acid (HOCl). Sometimes these molecular species act as source of ROS.

**REACTIVE OXYGEN SPECIE (ROS) IN NORMAL PHYSIOLOGY**

Normally, low concentration of ROS is essential for normal physiological functions like gene expression, cellular growth and defense against infection. Sometimes they also act as the stimulating agents for biochemical processes within the cell (Droge, 2002). ROS exert their effects through the reversible oxidation of active sites in transcription factors such as nuclear factor-kappa B (NF-kB) and activator protein-1 (AP-1) leading to gene expression and cell
growth (Schreck and Baeuerle, 1991). ROS can also cause indirect induction of transcription factors by activating signal transduction pathways (Schreck and Baeuerle, 1991). One example of signal transduction molecules activated by ROS is the mitogen activated protein kinases (MAPKs). ROS also appear to serve as secondary messengers in many developmental stages. For example, in sea urchins ROS levels are elevated during fertilization. Similarly prenatal and embryonic development in mammals has also been suggested to be regulated by ROS (Schreck and Baeuerle, 1991). ROS are also used by the immune system. For example, ROS have been shown to trigger proliferation of T cells through NF-κB activation. Macrophages and neutrophils generate ROS in order to kill the bacteria that they engulf by phagocytosis. Furthermore, tumor necrosis factor (TNF-α) mediates the cytotoxicity of tumor and virus infected cells through ROS generation and induction of apoptosis (Droge, 2002).

**REACTIVE OXYGEN SPECIES (ROS) INDUCED OXIDATIVE DAMAGE**

Reactions of biomolecules such as lipid, protein and DNA with ROS depending upon their nature, produce different types of secondary radicals such as lipid radicals, sugar and base derived radicals, and amino acid radicals. These radicals in presence of oxygen are converted to peroxyl radicals. Peroxyl radicals are critical in biosystems, as they often induce chain reactions. The biological implications of such reactions depends on several factors like site of generation, nature of the substrate, activation of repair mechanisms, redox status among many others.

For example, cellular membranes are vulnerable to the oxidation by ROS due to the presence of high concentration of unsaturated fatty acids in their lipid components. ROS reactions with membrane lipids cause lipid peroxidation, resulting in formation of lipid hydroperoxide (LOOH) which can further decompose to an aldehyde such as malonaldehyde, 4-hydroxy nonenal (4-HNE) or form cyclic endoperoxide, isoprotans, and hydrocarbons. The consequences of lipid peroxidation are cross linking of membrane proteins, change in membrane fluidity and formation of lipid-protein, lipid-DNA adduct which may be detrimental to the functioning of the cell.
ANTIOXIDANT
Cells are usually equipped with an impressive repertoire of antioxidant enzymes as well as small antioxidant molecules. These agents may not be sufficient enough to normalize the redox status during oxidative stress. Under such conditions, supplementation with exogenous antioxidants is required to restore the redox homeostasis in cells. Recent epidemiological studies have shown an inverse correlation between the levels of established antioxidants (vitamin E and C), phytonutrients present in tissue, blood samples and cardiovascular disease, cancer and with mortality due to these diseases. Since several plant products are rich in antioxidants and micronutrients, it is likely that dietary antioxidant supplementation protects against the oxidative stress that mediated disease development. Therefore, to maintain optimal body function, antioxidant supplementation has become an increasingly popular practice.

NATURAL ANTIOXIDANTS
A variety of dietary plants including grains (cereals), legumes, fruits, vegetables, tea, wine etc. contain antioxidants. The prophylactic properties of dietary plants have been attributed to the antioxidants is the polyphenols present in them. Polyphenols with over 8000 structural variants are secondary metabolites of plants and represent a huge gamut of substances having aromatic ring(s) bearing one or more hydroxyl moieties. Polyphenols are effective ROS scavengers and metal chelators due to the presence of multiple hydroxyl groups. Examples of polyphenolic natural antioxidants derived from plant sources include vitamin E, flavonoids, cinnamic acid derivatives, curcumin, caffeine, catechins, gallic acid derivatives, salicylic acid derivatives, chlorogenic acid, resveratrol, folate, anthocyanins and tannins (Bors et al, 1996). Aside polyphenols, there are also some plant derived non-phenolic secondary metabolites such as melatonin, carotenoids, retinal, thiols, jasmonic acid, eicosapentaenoic acid, ascoipyrones and allicin that show excellent antioxidant activity. Ascorbate (vitamine C), the water soluble natural vitamin, plays a crucial role in regenerating lipid soluble antioxidants like vitamin E. Vitamin E and C are used as standards for evaluating the antioxidant capacity of new molecules.

MAJOR FUNCTIONS OF THE LIVER
The most important functions of the liver are:
- Uptake of nutrients supplied by the intestines via the portal vein.
- Biosynthesis of endogenous compounds and storage, conversion, and degradation of them into excretable molecules (metabolism). In particular, the liver is responsible for the biosynthesis and degradation of almost all plasma proteins.
- Supply of the body with metabolites and nutrients.
- Detoxification of toxic compounds by biotransformation.
- Excretion of substances with the bile.

HEPATOTOXICITY
Liver is the most important organ that plays an important role of detoxification and also is the first victim of toxins leading to hepatotoxicity. Most of the liver damages are induced by lipid peroxidation and other oxidative damages caused by toxins. Exogenous factors like smoke, stress, alcohol, fatty food will trigger free radical generation which further leads to mucosal ischemia, excess secretion of hydrochloric acid, pepsin, ultimately resulting in ulcer. In India, more than 93 medicinal plants are used in different combination in the preparation of about forty patented herbal formulations. Plants with antioxidant potential as major mechanism along with other mechanisms are usually used for liver and gastric protection. Smooth endoplasmic reticulum of the liver is the principal ‘metabolic clearing house’ for both endogenous chemicals like cholesterol, steroid hormones, fatty acids and proteins, and exogenous substances like drugs and alcohol. Liver plays a central role in the metabolism and excretion of xenobiotics which makes it highly susceptible to their adverse and toxic effects (Saukkonen et al, 2006).

Hepatotoxicity refers to liver dysfunction or liver damage that is associated with an overload of drugs or xenobiotics (Navarro et al, 2006). The chemicals that cause liver injury are called hepatotoxins or hepatotoxicants. Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals, natural chemicals like microcystins, herbal remedies and dietary supplements (Willet et al, 2004; Papay et al, 2009). 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). 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). Hepatotoxicity related symptoms may include;
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 and Schaino, 2007)

Common Medicinal Plants Having Hepatoprotective Activity.

Table 1.0

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant</th>
<th>Active component(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Achillea millefolium (Gandana, Biranjasipha)</td>
<td>Caffeic acid</td>
</tr>
<tr>
<td>2</td>
<td>Andrographis paniculata (Kalmegh)</td>
<td>Andrographolide</td>
</tr>
<tr>
<td>3</td>
<td>Anoectochilus formosanus</td>
<td>Kinsenoside</td>
</tr>
<tr>
<td>4</td>
<td>Bacopa monniera (Bramhi)</td>
<td>Bacoside A</td>
</tr>
<tr>
<td>5</td>
<td>Cassia tora (Puvad, Chakvd)</td>
<td>Ononitol monohydrate</td>
</tr>
<tr>
<td>6</td>
<td>Cassia fistula (Amaltas)</td>
<td>Ethanolic extract</td>
</tr>
<tr>
<td>7</td>
<td>Cichorium intybus (Kasni, Chicory)</td>
<td>Alcoholic extract, flavonoids</td>
</tr>
<tr>
<td>8</td>
<td>Colchicum autumnale (Suranjan)</td>
<td>Cochinine</td>
</tr>
<tr>
<td>9</td>
<td>Curcuma longa (Haridra, turmeric)</td>
<td>Curcumin</td>
</tr>
<tr>
<td>10</td>
<td>Eclipta alba (Bhringaraj)</td>
<td>Ethanolic extract</td>
</tr>
<tr>
<td>11</td>
<td>Equisetum arvense (Horsetail)</td>
<td>Onitine, Kaempferol-3-o-glucoside</td>
</tr>
<tr>
<td>12</td>
<td>Foeniculum vulgare (Mishreya, Fennel)</td>
<td>Essential oil</td>
</tr>
<tr>
<td>13</td>
<td>Garcinia mangostana (Vrikshamla)</td>
<td>Garcinone E</td>
</tr>
<tr>
<td>14</td>
<td>Glycyrrhiza glabra (Yashti-madhu, Licorice)</td>
<td>Glycyrrhzin</td>
</tr>
<tr>
<td>15</td>
<td>Jatropha curcas (Ratanjyot Jangli erandi)</td>
<td>Methanolic extract</td>
</tr>
<tr>
<td>16</td>
<td>Phyllanthus amarus (Bhuiamala)</td>
<td>Lignans, alkaloids, bioflavonoids</td>
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<tr>
<td>17</td>
<td>Picrorhiza kuroa (Katuka)</td>
<td>Iridoid glycoside mixture (Picroliv)</td>
</tr>
<tr>
<td>18</td>
<td>Protium heptaphyllum (Almecega)</td>
<td>α- And β- Amyrin</td>
</tr>
<tr>
<td>19</td>
<td>Silybum marianum (Milk thistle)</td>
<td>Flavonolignan (Silymarin)</td>
</tr>
<tr>
<td>20</td>
<td>Solanum nigrum (Makoi)</td>
<td>Aqueous extract</td>
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<td>21</td>
<td>T. catappa (Jangli badam)</td>
<td>Punicalagin and punicalin</td>
</tr>
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<td>22</td>
<td>Trigonella foenum graecum (Chandrika)</td>
<td>Polyphenolic extract</td>
</tr>
<tr>
<td>23</td>
<td>Wedelia calendulae (Peela Bhangra)</td>
<td>Alcoholic extract</td>
</tr>
</tbody>
</table>

BACKGROUND OF THE STUDY
Exposure to toxic chemicals, environmental pollutants and drugs can cause cellular injuries through metabolic activation of reactive oxygen species. The present study tested the hypothesis that extract of *G. albidum* may have hepatoprotective effect.

OBJECTIVE OF THE STUDY
The objective of this work is to evaluate the lipid profile and hepatoprotective effect of oral administration of methanol extract of *Gambia abidium* (udara) in wistar albino rats.

MATERIALS AND METHODS

EQUIPMENT
Refrigerator: Super power refrigerator. Made by Phyilco-company limited.
Centrifuge: Hettich universal centrifuge. manufactured by Andreas Hettich, Tuttingen, Germany.

CHEMICALS
Assay kits for the estimation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, HDL-Cholesterol, LDL-Cholesterol, Total Cholesterol, and Triglyceride were purchased from Randox, UK. All other chemicals were of analytical grade.

PLANT MATERIAL
Fresh leaves of the plant *Gambia albidum* were obtained from a local farm in Umuariaga 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 to powdery form.
PLANT EXTRACTION
The powdered leaves of *G. albidum* were 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.

ANIMALS
Healthy female wistar albino rats of 130 gram mean weight were used for the study. All animals were kept in the 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*.

EXPERIMENTAL DESIGN
Sixteen female albino wistar rats were used for this study. Animals were grouped into four; A, B, C, and D. Group (A) and (B) were the test groups. Group (C) and (D) were the control groups, each group having four rats. Test Groups A and B were given 250 and 500mg/kg body weight of *G. albidum* leave extract respectively for 10 days orally. Group (C) represented the negative control group and Group (D) represented the positive control group. All the rats used in this study were initially subjected to hepatocyte damage using 2.0 ml of hexane/chloroform in the ratio of 4:6 except the normal control group.

EVALUATION OF THE VARIOUS PARAMETERS STUDIED
DETERMINATION OF REDUCED GLUTATHIONE (GSH)
Reduced glutathione (GSH) was determined by the method of Ellman (1959).
To 0.1 ml of sample, 2.4 ml of 0.02M EDTA solution was added and kept on ice bath for 10min. Then 2 ml of distilled water and 0.5 ml of 50% TCA were added. This mixture was kept on ice for 10-15 min and then centrifuged at 3000 rpm for 15 min. 1 ml of was taken out and 2ml of Tris-Hcl buffer was added. Then 0.05 ml of DTNB solution (Ellman’s reagent) was added and vortexted thoroughly. OD was read (within 2-3min after the addition of DTNB) at 412 nm against a reagent blank. Absorbance values were compared with a standard curve generated from known GSH.

DETERMINATION OF VITAMIN C
Determination of ascorbic acid was according to the method proposed by Emadi *et al.*, (2005). A portion of the sample (1.0ml) was placed into a small test tube. Then 1.0ml of 10% trichloroacetic acid was added followed by addition of 0.5ml of chloroform.
The reaction was stopped and the test tube shook for 15 seconds and centrifuged at 3,000 rpm. A portion of the clear supernatant (1.0 ml) was placed in another test tube. Blank and standard was prepared by adding 0.5 ml of 10% trichloroacetic acid to 0.5 ml and working standard reagent. To each test tube was added 0.4 ml freshly prepared combine color reagent. The tubes were stopped and placed in a water bath at 56°C for one hour. They were cooled in the ice bath for about 5 mins. To each test tube was added, slowly with mixing, 2.0 ml of ice cold 85% sulphuric acid. The tubes were left at room temperature for 30 mins, then mixed and the optical density (OD) read in the colorimeter at 490 nm using blank to zero the instrument. The OD of the standard was also read.

**ASSAY FOR ALANINE AMINOTRANSFERASE**

Determination of alanine aminotransferase was carried out according to Reitman and Frankel (1957) method.

A portion (0.1 ml) of the sample was put into a test tube followed by introduction of 0.5 ml of the reagent (Sodium azide) into same test tube using a pipette.

It was mixed and incubated at exactly 30 mins at 37°C. A small portion (0.5 ml) was collected and mixed with 2,4-dinitrophenylhydrazine and allowed to stand for exactly 20 mins at 25°C. 5.0 ml of Sodium hydroxide was added and mixed with the solution. Absorbance was read of the sample at 546 nm against the sample blank.

**ASSAY FOR ASPARTATE TRANSAMINASE**

Determination of aspartate aminotransferase was carried out according to Reitman and Frankel (1957) method. (Measurement against reagent blank).

A portion (0.1 ml) of the sample was put into a test tube followed by introduction of 0.5 ml of the reagent (Sodium azide) into same test tube using a pipette. To the second test tube, 0.5 ml of reagent (Sodium azide) and 0.1 ml of distilled water was put. It was mixed and incubated at exactly 30 mins at 37°C. Then, 0.5 ml of the second reagent (2,4-dinitrophenylhydrazine) was put, mixed and allowed to stand for exactly 20 mins at 25°C. A portion (5.0 ml) of sodium hydroxide was mixed and absorbance of sample read against the reagent blank after 5 mins at 546 nm.
CATALASE ASSAY
Determination of catalase activity was according to Aebi (1974) method.
A portion (2.27 ml) of hydrogen peroxide was made up to 100ml with water. 10mls of 5% potassium dichromate, 20 ml of glacial acetic acid, 2mls of the hydrogen peroxide and 2.5mls of phosphate buffer was added to 0.5ml of sample and mixed.

A portion (1ml) of the reaction mixture was added to dichromate acetic acid reagent. Absorbance was read at 570nm.

SUPEROXIDE DISMUTASE (SOD)
This was determined using the method Xin et al. (1991).
A portion of adrenalin (0.01g) was dissolved in 17 ml of distilled water. A portion (0.1 ml) of the sample and 0.9mls of phosphate buffer were mixed. A portion (0.2ml) was taken in triplicate and 2.5ml of buffer. Inside the cuvette, 3ml of adrenalin solution was mixed and absorbance read at 480nm at 30sec interval for five times.

LIPID PEROXIDATION (MALONDIALDEHYDE-MDA)
Lipid peroxidation was determined spectrophotometrically by measuring the level of lipid peroxidation product, malondialdehyde (MDA) as described by Wallin et al (1993).

To a test tube, 0.1ml of sample was added followed by 0.9 ml of distilled water, 0.5 ml of 25% TCA and 0.5ml of 1% TBA in 0.3% NaOH was added. It was incubated at 95°C for 40mins and then cooled in water. A portion (0.1ml) of sodium dodecyl sulphate was added. Absorbance was read at 532 and 600nm against a blank.

LOW DENSITY LIPOPROTEIN (LDL).
Low density lipoprotein was determined according to Bergmenyer (1985).

Precipitation reaction
A portion of the precipitant solution (0.1ml) was added into 0.2ml of sample and allowed to stand for 15mins at room temperature (20-25°C). It was centrifuged at 2,000 ×g for 15 min. The cholesterol concentration in the supernatant was determined.

Cholesterol assay
The concentration of the serum Total Cholesterol was determined according to the QCA CHOD-PAP method.
CALCULATIONS

LDL- Cholesterol (mg/dl) = Total Cholesterol (mg/dl) – 1.5 × supernatant Cholesterol (mg/dl).

DETERMINATION OF TOTAL CHOLESTEROL

Total cholesterol was determined according to Abell et al, 1952.

A portion of the sample (10µl) was mixed with 1000µl of reagent (4-aminoantipyrine, phenol, peroxidase, Cholesterol esterase, Cholesterol oxidase, Pipes buffer) and incubated for 10mins at 25°C.

Absorbance of the sample was read against reagent blank within 60minutes at 546nm.

DETERMINATION OF HIGH DENSITY LIPOPROTEIN (HDL) CHOLESTEROL

HDL-Cholesterol was determined using Dextran sulphate method. (Alber et al, 1978).

To a test tube, 0.3ml of sample was added using pipette, followed by the addition of a drop of HDL working reagent (10g/L of Dextran sulphate + 1m of magnesium acetate). It was properly mixed and incubated at 25°C for 15mins, followed by centrifugation at 2500 rpm for 15mins. The supernatant was used to determine the concentration of HDL-cholesterol. Three test tubes were selected and labeled standard, sample and blank. In the standard test tube, 0.01ml of cholesterol working reagent was added. In the sample test tube, 0.01ml of the supernatant and 1.00mL of cholesterol working reagent were put and mixed well. In the blank test tube, 0.01mL of distilled water was used. The test tubes were incubated at 37°C for 15mins and the absorbance read at 546nm.

DETERMINATION OF TRIACYGLYCEROL

Triglyceride was determined using enzymatic test glycerol-phosphate oxide method (Jacobs et al, 1960).

Three test tubes were selected and labeled blank, standard and sample. In the standard test tube, 0.01mL of standard was put and 1.00ml of triglyceride working reagent (40mM of pipes buffer, pH 7.5, 6mM of ATP, 5mM of Mgcl2, 400µ/L of glycerol kinase and 155µ/L of glycerol 3- phosphate oxidase). In the sample test tube, 0.01mL of sample and 1.00mL of triglyceride working reagent were put and mixed and in the blank test tube, 0.01mL of distilled water was put using a pipette. The test tubes were incubated at 37°C for 5mins and the absorbance read at 546nm.
DETERMINATION OF SERUM TOTAL PROTEIN
Serum total protein was determined by the biuret reaction as proposed by George, 1939.
To exactly 4 cc. of 10 per cent sodium hydroxide in a photoelectric calorimeter tube, 0.1 cc. of fresh serum (free from cells) was added with a folin micropipette. The pipette was rinsed out three times with sodium hydroxide solution and mixed by rotating, and then 0.5 cc. of 1 per cent copper sulfate was added. It was shook vigorously five times. It was allowed to stand for 25 minutes and read in a photoelectric calorimeter.

DETERMINATION OF ALBUMIN
Albumin was determined according to Grant et al (1987) method.
A portion of the sample (0.01 ml) and 3.00ml of BCG reagent was put into a test tube, mixed and incubated at 25°C. The absorbance of the sample and that of the standard against the reagent blank was determined using a spectrophotometer at 630nm.

DETERMINATION OF VITAMIN E (α-TOCOPHEROL)
Vitamin E analysis was done according to Wei method (2011).
A portion (150 mg) of Na₂SO₄ was placed in a test tube and put in a desiccator to avoid moisture. About 100 µL of the sample was put into the test tube using an automatic pipette and the tip of the pipette rinsed with distilled water each time after use. A portion (2 ml) of acetone was added and the mixture shook vigorously to denature the proteins. About 500 µL internal standard solution was added to the test tube, shook, and allowed to stand for 2 hrs. The test tube was centrifuged for 20 mins. Using a pasture pipette the supernatant was collected and transferred to a syringe connected to a 0.45 µm filter. The test tube was rinsed with 1.5 mL of acetone and transferred into the syringe and filtered again. The mixture was evaporated under gently at 40°C. The dry tube was capped and put in ice for 2 mins. Cm (1:3 w/BHT) mixture flask was also put in the ice. 500µL of CM mixture was added into another test tube and the tube was vortexed 3times. The tube was rapped and placed to stand for 2 hrs in the freezer. A portion (50µL) of the final solution was injected into the HPLC system and ran for 6mins 30secs. Degased methanol was used as the mobile phase with a flow rate of 2mL/min. Detection was monitored at 292nm. The linear range of α-tocopherol standard curve was from 50-500ng (r = 0.999).
RESULTS

The data are expressed as quadruplet mean ± standard deviation using bar charts. Comparisons were made between the two tests groups treated with different doses of the extract: 250mg representing the low dosage and 500mg representing the high dosage with the normal control, and negative control groups. These were performed using the paired T-Test. The significance was accepted at p < 0.05.

4.2.1 EFFECT OF METHANOL EXTRACT OF GAMBIA ALBIDUM ON AST

![Graph showing the effect of methanol extract of Gambia albidum on AST.](image)

From fig4.1 above, the mean value of the lower dose increased significantly compared to the normal control and the negative control groups at (p< 0.05).

The mean value of higher dose however increased but not significantly when compared to the negative control group. The mean value of the high dose group increased significantly (p< 0.05) when compared with the normal control group.

4.2.2 EFFECT OF METHANOL EXTRACT OF GAMBIA ALBIDUM ON ALT

![Graph showing the effect of methanol extract of Gambia albidum on ALT.](image)
In fig 4.2 above, the mean values of the high and low dose group increased when compared against the normal and negative control groups but not significant (p > 0.05)

**4.2.3 EFFECT OF METHANOL EXTRACT OF GAMBIA ALBIDUM ON T.CHOL**

![Graph showing the effect of Gambia Albidum on T.Chol](image)

**Fig 4.3**

From fig 4.3 above, mean value of the low dosage group increased but non-significant when compared to the normal control group. Significant increase was observed when the low dosage group is compared to the negative control.

The mean values of the high dosage group increased but not significant compared to both the normal and negative control groups.

**4.2.4 EFFECT OF METHANOL EXTRACT OF GAMBIA ALBIDUM ON HDL**

![Graph showing the effect of Gambia Albidum on HDL](image)

**Fig 4.4**
In fig 4.4 above, the low and high dosage groups showed increased mean values compared to both the normal and the negative control groups but not significant.

4.2.5 EFFECT OF METHANOL EXTRACT OF *GAMBIA ALBIDUM* ON TAG

![Graph showing TAG levels for different groups](image)

Fig 4.5

In fig 4.5 above, the mean value of the low dosage group showed significant decreased than the normal and negative control groups (p< 0.05).

The mean value of the high dosage group decreased when compared against the normal control group but not significant. The decrease shown when the mean value of the high dosage group is compared to the negative control was significant (p< 0.05).

4.2.6 EFFECT OF METHANOL EXTRACT OF *GAMBIA ALBIDUM* ON LDL

![Graph showing LDL levels for different groups](image)

Fig 4.6
In fig 4.6 above, the mean values of the low and high dosage groups increased compared to the normal and negative control but not significant (p> 0.05).

4.2.7 EFFECT OF METHANOL EXTRACT OF GAMBIA ALBIDUM ON MDA

![Graph](https://via.placeholder.com/150)

Fig 4.7

In fig 4.7 above, the mean value of the low dosage group from the above chart increased significantly (P< 0.05) when compared against the positive and negative control groups.

The mean value of the high dosage group also increased when compared to the normal and negative control groups but not significant (P> 0.05).

4.2.8 EFFECT OF METHANOL EXTRACT OF GAMBIA ALBIDUM ON GSH

![Graph](https://via.placeholder.com/150)

Fig 4.8

In fig 4.8 above, the mean value of the low dosage group increased non-significantly when compared to the normal control group, but decreased significantly (p< 0.05). When compared to the negative control group.
The mean value of the high dosage group also increased non-significantly when compared to the normal control group, but decreased significantly (p<0.05) when compared to the negative control group.

**4.2.9 EFFECT OF METHANOL EXTRACT OF *GAMBIA ALBIDUM* ON VIT.E**

![Vitamin E Graph](image)

**Fig 4.9**

In fig 4.9 above, the mean values of the low and high dosage groups non-significantly (p>0.05) decreased when compared to both the normal and negative control groups.

**4.2.10 EFFECT OF METHANOL EXTRACT OF *GAMBIA ALBIDUM* ON VIT.C**

![Vitamin C Graph](image)

**Fig 4.10**

In fig 4.10 above, the mean value of the low dosage group decreased non-significantly when compared to the normal control group, but increased non-significantly (p>0.05) when compared to the negative control group.
The mean value of the high dosage group also decreased non-significantly when compared to the normal control, but increased non-significantly when compared to the negative control group.

4.2.11 Effect of Methanol Extract of Gambia Albidum on T.Protein

![T.Protein Graph]

Fig 4.11

In fig4.11 above, the mean value of the low dosage group increased non-significantly (p > 0.05) compared to the normal and negative control groups.

There was a mean value decrease in the high dosage group when compared to the normal and negative control groups.

4.2.12 EFFECT OF METHANOL EXTRACT OF GAMBIA ALBIDUM ON ALBUMIN

![Albumin Graph]

Fig 4.12
In fig 4.12 above, the mean value of the low dosage group increased when compared to the normal and negative control groups but not significant (P< 0.05).

The mean value of the high dosage group on the other hand decreased though not significant (p< 0.05) compared to the normal and negative control groups.

**4.2.13 EFFECT OF METHANOL EXTRACT OF *GAMBIA ALBIDUM* ON CAT**

![Graph showing CATALASE levels for different groups](image)

**Fig 4.13**

In fig 4.13 above, the mean value of the low dosage group decreased significantly (p< 0.05) when compared to the normal control group, but no significant decrease compared to the negative control group.

The mean value of the high dosage group decreased compared to the normal control, but had a significant (p< 0.05) decrease compared to the negative control group.

**4.2.14 EFFECT OF METHANOL EXTRACT OF *GAMBIA ALBIDUM* ON SOD**

![Graph showing SOD levels for different groups](image)

**Fig 4.14**
In fig4.14 above, the mean value of low dosage group decreased but not significant (p< 0.05) compared to the normal group, but non-significantly increased compared to the negative control.

The mean value of the high dosage group decreased compared to the normal and negative control groups, but not significant (p< 0.05).

**DISCUSSION**

Liver plays a central role in the metabolism and excretion of xenobiotics which makes it highly susceptible to their adverse and toxic effects. Liver injury caused by various toxic chemicals or their reactive metabolites (hepatotoxicants) is known as hepatotoxicity. The hepatotoxins produce a wide variety of clinical and histopathological indicators of hepatic injury. Liver injury can be diagnosed by certain biochemical markers like alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Elevations in serum enzyme levels are taken as the relevant indicators of liver toxicity (Reuben, 2004). In this work, the effect of the extract of *Gambia albibum* on the AST serum level in groups (A) and (B) showed a significant (p<0.05) increase compared to the normal control (group D). With increasing dose, AST showed reduction in value. While the difference in mean values of AST of test groups (A) and (B) compared to the negative control (group C) was not significant. The ALT levels in the two test groups also increased compared to the normal control group though non-significant at (p>0.05). With increasing dose, ALT was lowered close to normal. The ALT level in test groups (A) and (B), and the negative control showed non-significant difference. Significant increases in liver enzymes suggest liver damage. Liver enzyme levels may reflect the extent of hepatocellular necrosis but may not correlate with eventual outcome. In fact, declining AST and ALT may indicate either recovery or poor prognosis in fulminant hepatic failure according to the studies of Friedman (2003).

Living systems are protected from ROS by antioxidant enzymes (SOD, CAT, GSH etc.) and other endogenous antioxidant sources (Nilesh et al, 2010). Studies by Curtis and Mortiz, (1972) showed SOD as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Chelikani et al, 2004). SOD level in the test groups decreased compared to normal control which is an indication of liver...
damage. SOD level showed increased mean value close to normal with increasing dose. Group (A) showed elevated SOD values compared to the negative control which agrees with studies of Onyeka et al, (2012). Catalase (CAT) level was significantly lower than normal in Group (A). Group (B) showed an increase in CAT activity close to normal which is an indication of the antioxidant property of the extract. This agrees with previous study by Abiodun et al, (2011).

MDA is a product of lipid peroxidation (Devaki et al, 2004). Extensive lipid peroxidation leads to disorganization of membrane by peroxidation of unsaturated fatty acids which also alters the ratio of polyunsaturated to other fatty acids. This would lead to a decrease in the membrane fluidity and the death of the cell (Devaki et al, 2004). In this study, MDA level showed a dose-dependent decrease close to normal level. This implies that *G. albidum* extract could have antioxidant properties by decreasing lipid peroxidation. This is consistent with earlier studies by Abiodun et al, (2011).

The activity of GSH in Groups (A) and (B) showed a dose-dependent increase compared to the normal control. This shows the ability of the leaf extract in increasing the competency of liver in detoxification of xenobiotics, as GSH is a major detoxifier in the liver. This finding also agrees with studies by Abiodun et al, (2011).

The liver is the major source of most of the serum proteins. The parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors and most of the ‘a’ and ‘b’ globulins (Forman and Barnhart, 1964). The estimation of total proteins in the body is helpful in differentiating between a normal and damaged liver, as the majority of plasma proteins like albumins and globulins are produced in the liver (Thapa and Walia, 2007). Total protein is often reduced slightly but the albumin to globulin ratio shows a sharp decline during hepatocellular injury (Thapa and Walia, 2007). In this work, the effect of the leaves of *Gambia albibum* on the serum levels of total protein in albino rats was also studied. The mean value of total protein in group (A) non-significantly increased compared to the normal. Group (B) total protein non-significantly decreased compared to the normal. Test on albumin level showed that Group (A) increased non-significantly compared to the normal. Group (B) decreased but also non-significantly compared to the two control groups. This study shows that protein level decreased with increasing level of extract.
From the result of this study, total cholesterol level in Group (A) was higher than the normal control which decreased with increasing dose. The HDL levels in the test groups, A and B showed a dose-dependent increase compared to the normal and negative control groups. While LDL level showed a dose dependent decrease compared to group D (normal control). The TAG level in Group (A) and (B) showed a dose dependent decrease compared to the normal control group. TAG Level in test groups (A) and (B) showed a dose dependent increase compared to group C (negative control).

Vitamin C (ascorbate) level in group (A) and (B) increased compared to group C (negative control) indicating antioxidant and hence, hepatoprotective effect of *G.albidum* extract.

Vitamin E (α-tocopherol) activity in the study showed a dose-dependent increase close to group D (normal) values. Again, indicating the ability of the *G. albidum* to act as an antioxidant supplement.

**CONCLUSION**

The present investigation of the effect of methanol extract of *Gambia albidum* against liver cell damage and lipid profile in albino rats showed that *G. albidum* has antioxidant properties indicated by decrease in lipid peroxidation, increasing ascorbate and α-tocopherol activity in a dose-dependent manner and increasing the serum antioxidant enzyme levels. This study revealed that administration of *G. albidum* may also be useful in the treatment and prevention of hepatic stress as demonstrated by decrease in levels of liver damage marker enzymes with increasing dose of the extract. It also shows that *G. albidum* may decrease the risk of heart attack as indicated by the dose-dependent increase in HDL level and decrease in LDL and total cholesterol levels.

**REFERENCE**

