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HEPATOPROTECTIVE AND RENAL EFFECTS OF METHANOL EXTRACT OF*ALBUCA NIGRITANA* BULB IN FEMALE WISTAR RATS

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ABSRACT

Methanol extract of *Albuca nigritana* bulb was investigated for its hepatoprotective and renal effects on hexane/chloroform induced hepatocellular damage in female wistar rats. The plant extract was administered at 250mg/kg and 500mg/kg body weight to the test groups randomized into two groups. Liver enzyme markers and important biochemical parameters were measured and compared with the positive control group. The plant extract showed no hepatoprotective effect but adverse effect on renal function as judged by the level of antioxidants, liver marker enzymes, urea, creatinine and electrolyte level in the sera of the induced experimental animals. The AST level showed a significant increase at 250mg/kg dose of the extract ($p < 0.05$) the level of ALT was not significantly increased ($p>0.05$), the level of total protein was not significantly reduced ($p>0.05$) and the albumin level was not significantly increased ($p>0.05$). The urea, creatinine and MDA levels were significantly elevated ($p < 0.05$). Also there was also a significant reduction in glutathione, catalase, and vitamin C and E levels ($p<0.05$), while only SOD showed a significant increase at a dose of 500mg/kg of plant extract ($p<0.05$). Finally Na^+ and Cl^- showed a significant increase in the serum level ($p <$

0.05). Only potassium showed a non-significant increase ($p>0.05$). It was therefore concluded that *Albuca nigritana* has no hepatoprotective effect but adverse renal effect on the animals used for the study.

KEYWORDS: *Albuca nigritana,* hepatoprotective effect.

INTRODUCTION

The liver is an important organ of the body that regulate homeostasis; it is largely made up of hepatocyte cells which make up 70-85% of the liver cytoplasmic mass. These cells are involved in: protein synthesis, protein storage, transformation of carbohydrates, and synthesis of cholesterol, bile salt and phospholipids. Detoxification, modification and excretion of exogenous and endogenous substances. Formation and excretion of bile are the other important functions of hepatocytes (Ward et al., 1991).

The liver in addition to performing physiological functions also protects the body cells against toxic effect of harmful drugs and chemicals. Over the years, the problem of liver damage and disease has been on the increase owing to the rapid urbanization which promotes the release of toxic substances that has a continuous effect on the liver. Following this, several researches has been going on in the field of hepatology in a view to finding a lasting solution to hepatocyte damage and hence find a better way of protecting the liver against these damages.

Liver injury or liver dysfunction has been a major health problem that challenges not only healthcare professionals but also the pharmaceutical industries and drug regulatory agencies. Liver cell injury caused by various toxic chemicals like certain antibiotics, chemotherapeutic agents, carbon tetrachloride (CCl4), thioacetamide (TAA), other haloalkanes, excessive alcohol consumption and microbes is well studied.

Tremendous scientific advancement has been made on in this regard. In spite of this, liver problems have been on the increase (Pang et al.,1992). Presently, a few hepatoprotective drugs that are gotten from natural sources are available for treatment of liver disorders. Hence, attention has been diverted towards the use of traditional systems of medicine for remedies to hepatic disorders.

Albuca nigritana plant commonly called Nigerian squil is a plant that belongs to the family of *Hyacinthaceae. Albuca nigritana* are mostly found in damp places. They produce green

spindle-like leaves that form a bulb; the leaves are of different shades of green. *Albuca nigritana* has been shown to contain some important phytochemicals and as such possess antibacterial properties. However, to the best of my knowledge there are no reported works on the antioxidant property of this plant. Hence this work is set to investigate the antioxidant properties as well as hepatoprotective and renal effects of *Albuca nigritana* bulb methanol extract on hexane-chloroform induced hepatocyte damage on female wistar rats.

OBJECTIVE OF STUDY

The aim of this study is to:

1. To investigate the hepatoprotective effect of *Albuca nigritana* plant extract on female albino wistar rat induced with hepatocellular damage using important enzyme markers and metabolites as parameters.
2. To investigate the renal effect of the plant extract on albino wistar rats using important metabolites as markers.
3. To investigate the efficiency of *Albuca nigritana* plant as a hepatoprotective medicine

In addition, the protective effect of ethanol extract of *Sargassum polycystum* was evaluated in D-galactosamine-induced hepatitis in rats, and has demonstrated antioxidant activity against D-galactosamine-induced hepatitis by inhibiting the activation of lipid peroxidation and by preserving the hepatic enzymatic and non-enzymatic antioxidant defence system at near normal.

Finally methanol, hexane and chloroform extracts of *Prostechea michuacana* were studied against CCl_4-induced hepatic injury in *albino* rats. Pre-treatment with methanol extract reduced biochemical markers of hepatic injury levels demonstrating dose-dependent reduction in the in vivo peroxidation induced by CCl_4. Likewise, pre-treatment with extracts of *P.michuacana* on paracetamol-induced hepatotoxicity and the possible mechanism involved in this protection were also investigated in rats after, the degree of protection was measured by monitoring the blood biochemical profiles. The methanol extract of orchid produced significant hepatoprotective effect as reflected by reduction in the increased activity of serum enzymes, and bilirubin. These results suggested that methanol extract of *P.michuacana* could protect paracetamol-induced lipid peroxidation thereby eliminating the deleterious effects of toxic metabolites of paracetamol. This hepatoprotective activity was comparable with sylmarin. Hexane and chloroform extracts did not show any apparent effect.

The findings indicated that the methanol extract of P.michhuacana can be a potential source of natural hepatoprotective agent.

MATERIALS AND METHODS

MATERIALS

PLANT MATERIAL: *Albuca nigritana* bulb

PLANT COLLECTION AND IDENTIFICATION

The plant part was gotten from a settlement in Ohafia L.G.A of Abia state and was identified as *Albuca nigritana* bulb by Dr G. S Aloh of the department Biochemistry, Michael Okpara University of Agriculture, Umudike.

PLANT PRREPARATION

The bulbs were washed and prepared fresh. 100g of the bulbs were weighed out using a weighing balance and blended. 200ml of methanol was used to soak the prepared sample for 48hrs. The sample mixture was then extracted. The methanol extract was placed in a water bath to evaporate the methanol.

EXPERIMENTAL ANIMALS

Total of 16 female wistar rats were purchased from Vertinary department of University of Nigeria, Nsukka and were acclimatized for two weeks under normal temperature at the animal house of the department of Biochemistry, Michael Okpara University of Agriculture, Umudike. The animals were fed with normal feed and clean water for this period of weeks before administration of extract. The weights of the animals were taken at the end of the acclimatization period with the average of the rats standing at 127kg.

EXPERIMENTAL DESIGN

The 16 rats were randomized into four groups, groups A, B, C and D. Each group consist of four rats each.

Group A received feed and water and was referred to as positive control group.

Group B was induced with hepatocellular damage using chloroform and hexane in the ratio of 6:4 without treatment was referred to as negative control group.

Group C was induced with hepatocellular damage using chloroform and hexane in the same ratio as of group B above and was treated with 250mg/kg per body weight of the plant extract. This group was referred to as test group C.

Group D was induced with hepatocellular damage using Chloroform/hexane in the same ratio as of Group B and C above and was then treated with 500mg/kg body weight of plant extract. This group was referred to as test group D.

INDUCTION OF HEPATOCYTE DAMAGE

The rats in groups B, C and D were induced with liver damage by oral administration of 2ml of hexane/chloroform in the ratio of 4:6 and group C was left without treatment while groups B and C were treated with plant extract at different doses, (250mg/kg and 500mg/kg) per body weight respectively.

TREATMENT

Only group C and group D received treatment. Group C was treated with 250mg/kg of body weight of the plant extract while group D was treated with 500mg/kg. Group B was without treatment.

At the end of the experiment, the animals were sacrificed by cervical decapitation. Blood samples were collected, allowed to clot and centrifuged 2500rpm for 15min to get the serum and analysed for various biochemical parameters.

TEST

(A) ASSESMENT OF LIVER FUNCTION

The following biochemical parameters were used to asses liver function:

Alanine aminotransferase (ALT)

Aspartate aminotransferase (ALT)

Total protein

Total albumin

Catalase

Superoxide dismutase (SOD)

Reduced Glutathione (GSH)

Vitamin C

Vitamin E

Malondialdehyde (MDA)

(B) ASSESMENT OF RENAL FUNCTION

The following parameters were used to asses renal function

Urea

Creatinine

Electrolytes such as Na^+, K^+, Cl^-

METHODS

DETERMINATION OF SERUM CREATININE CONCENTRATION

Creatinine is derived from creatinine phosphate in muscle tissue and may be defined as a nitrogenous waste product. Creatinine is not reutilized but is excreted from the body in the urine through the kidney. Creatinine was measured to asses kidney function.

METHOD: spectrophotometric method described by Schirmeisteer et al (1964) and adopted by Bartel and Bormen (1972) was used in the determination of serum creatinine concentration.

REAGENTS: Standard, picric acid, sodium hydroxide etc.

PROCEDURE: Reagent blank, standard solution and sample solution were prepared. 50µl of H_2O was pipetting into a cuvette and mixed with 500µl of reagent to prepare reagent blank. Standard solution was prepared by pipetting 50µl of standard and mixing with 500µl reagent. Sample solution was prepared by pipetting 50µl of serum into a cuvette and mixed with 500µl of reagent. Their various absorbance were read at 500nm. 2ml reagent was then mixed with 0.2ml of standard solution and 2ml of reagent was also mixed with 0.2ml the sample solution and their absorbance A1 was read after 30sec. also 1.0ml of reagent was mixed with 0.1ml of standard solution and 0.1ml of sample was mixed 1.0 ml of reagent and the absorbance A2 was read.

DETERMINATION OF SERUM UREA CONCENTRATION

REAGENT: Urease, Sodium nitropuriside, phenol concentrate, Hypochlorite concentrate and standard.

PROCEDURE: Blank, standard solution and test solution were prepared. Blank was prepared by 50µl of reagent with 5µl of water. Standard solution was prepared by pipetting 5µl of sample into a cuvette and mixing with 50µl reagent and test solution was prepared by pipetting 5µl of serum into a cuvette and mixing with 50µl of reagent, and was incubated at

37^{oc} for 10min. 1.25 of reagent was again added to each mixture and their respective absorbance read at 570nm. In separate test tubes 10µl of distilled water was mixed with 100µl of reagent and 2.50ml of reagent added to for the blank preparation. The same was done with the standard and test solution and the absorbance (A) of sample and standard was read against the blank.

DETERMINATIONATION OF SERUM TOTAL PROTEIN CONCENTRATION

METHOD: Direct burette method (1968) was adopted.

REAGENTS: Sodium hydroxide, Potassium talitrate, preservatives and stabilizers.

PROCEDURES: Standard solution was prepared by measuring out 0.02ml of standard in a cuvette and mixed with water and left to stand for 10min. Test solution was prepared by measuring out 0.02ml of serum and mixed water. Their absorbance was read at 540nm against a reagent blank of 1ml of reagent.

SERUM ALBUMIN CONCENTRATION

METHOD: Method of Grant (1987) was adopted.

PROCEDURE: Reagent blank was prepared by mixing 3µl of H_2O WITH 100µl of reagent and incubated for 20min at 25^{oc}. Standard solution was prepared by mixing 3µl of standard with 1000µl of reagent and test solution was also prepared by mixing 3µl of serum with 1000µl of reagent and their absorbance was taken at absorbance within 60min. in separate test tubes, 0.01ml of standard (CAL) was mixed with 3.00ml of BCG reagent and allowed to incubate for 5min at 25^{oc}. The same was done for reagent blank test sample after which their respective absorbance was read against reagent blank.

DETERMINATION OF SERUM ALANIN AMINOTRANSFERASE CONCENTRAION (ALT TEST)

PRINCIPLE: Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenylhydrazin.

METHOD: The spectrophotometric method of Reitman and Frankel (1927) was adopted.

PROCEDURE: Reagent blank was prepared by measuring 0.5ml buffer into a test tube and 0.1ml of distilled water and allowed to incubate for 20min. 0.5ml of 2, 4 dinitrophenylhydrazine was then added and allowed to stand for 20min. 5.0ml of NaOH were added, mixed and incubated for 30min and absorbance was read at. The test solution was

prepared by pipetting 0.1ml of serum into a test tube and 0.5ml of reagent buffer was added and allowed to incubate for 30min at 37^{oc}. 0.5ml of 2,4 dinitrophenylhydrazine was then added and allowed to stand for 20min at 25oc. 5.0ml was finally added and absorbance sample was read against reagent blank after 5min.

DETERMINATION OF ASPARTATE AMINOTRANSFERASE (AST) CONCENTRATION

METHOD: Method of Reitman and Frankel (1957) was adopted and the same procedure follows that of ALT described above.

ESTIMATION OF MALONDIALDEHYDE (MDA) ACTIVITY

This was carried out to investigate lipid peroxidation level of the experimental animal.

METHOD: MDA activity was determined using spectrophotometric method as described by Wallin et al (1993).

REAGENT: Thiobarbituric acid, Sodium dodecyl sulphate, Sodium hydroxide.

PROCEDURE: To a test tube containing 0.3% NaOH, 0.1ml of serum was added in addition, 0.9ml of distilled water, 0.5ml of 1% TBA was also added. The test tube was then cooled and 0.1ml of 20% dodecyl sulphate was added an the absorbance was read at 540nm and 620nm against a blank. The values gotten from absorbance of 620nm (A629nm) were subtracted from Absorbance at 540nm (A540nm) and divided by a factor, 5.271 to give the value of MDA level in the serum.

ESTIMATION OF ANTIOXIDANT ACTIVITY

CATALASE ACTIVITY

METHOD: Catalase activity was determined using the method Aebi (1983).

REAGENT: Phosphate buffer, Hydrogen peroxide (H_2O_2)

PROCEDURE: 2.5ml of phosphate buffer was pipetted into a test tube; 2ml of H_2O_2 and 0.5ml of serum were added to it. 1ml portion of the mixture was put in another test tube and 2ml of dichromate acetic acid reagent was added and the absorbance was read at 240nm at a minute interval and catalase activity was calculated using the following equation:

ESTIMATION OF SUPERROXIDE DISMUTASE (SOD) ACTIVITY

METHOD: SOD activity was determined using a method described by Xin et al (1991)

REAGENT: Phosphate buffer, SOD standard, carbonate buffer, xanthine oxidase.

PROCEDURE: 100µL of serum was mixed with 800µl of distilled water to dilute it. 94µl of SOD standard and 1.25ml of carbonate buffer were added into a cuvette. 94µl of diluted serum was measured into a cuvette and 1.25 of carbonate buffer added. Their various readings were taken using a spectrophotometer at an absorbance of 500nm at room temperature. The changing rate of absorbance was used to determine superoxide dismutase activity. Concentration of superoxide dismutase in sample was determined by the comparison with the calibration curve from SOD standard.

ESTIMATION OF SERUM GLUTATHION CONCENTRATION

METHOD: The colorimetric method of determining glutathione was used.

PROCEDURE: 1ml portion of the test sample was mixed with 7ml portion of distilled water to prepare the test solution. The mixture was then treated with 2ml of distilled of 2-5 metaphospheric acid solution. After a minute it was centrifuged. The supernatant was used for the assay, while the extract in the test tube was added 2ml 0.5ml of phosphate buffer and was followed by the addition of 1ml of 0.5m NaOH, the absorbance was taken.

ESTIMATION OF VITAMIN C CONCENTRATION

Determination of vitamin C in the blood was determined using the method of the 2, 6 dichlorophenol indophenol colometric. A measured volume of the sample was mixed with 3ml of 3-6 metaphosphoric acid solution, mixed well and then centrifuged. 2ml of the supernatant was mixed with 0.5mls of 1.76 sodium citrate solution. Standards ascorbic acid was also prepared and diluted to a desired concentration using the same procedure for preparing test solution. A blank reagent was also prepared. The absorbance of the test solution and that of the standard solution was read against the blank at an absorbance of 520nm and Vitamin concentration was thus calculated.

DETERMINATION OF VITAMIN E CONCENTRATION

METHOD: the acid oxidative colorimetric method of the association of vitamin chemist was used.

Procedure: a measure of the serum sample was mixed with 5ml absolute alcohol followed by the addition of concentrated HNO_3, the mixture was left in a water bath at 90oc for 3 min, gradually cooled under running water. Then the volume was made up to 200ml in a standard flask.

A standard vitamin E solution was prepared as described above using Vitamin E standard and the absorbance of the sample solution and the standard solution was read against a reagent blank and vitamin E concentration calculated thus:

$$\text{Vitamin E mg/dl} = 100/v \times \frac{au \quad X \quad C}{av}$$

Where v = volume of sample

au = absorbance of sample

av = absorbance of standard

C = concentration.

TESTS FOR ELECTROLYTE ACTIVITY

ESTIMATION OF SODIIUM ION (Na^+) AND POTASSIUM ION (K^+)

METHODS: Flame photometric method was used

PROCEDURE: Stock sodium and Stock potassium were prepared. Stock sodium was prepared by dissolving 58.48g of sodium chloride in distilled water and made up of 1litre and stock potassium was prepared by dissolving 74.55g of potassium chloride in distilled water and made up to 1 litre.

Series of stock were made from these as follows:

Sodium/potassium (Mmol/litre)	Na^+ stock (ml)	K^+ stock (ml)	Distilled water (ml)
1. 120/2	120	2	878
2. 140/5	140	5	855
3. 160/8	160	8	832

Four test tubes were set up as follows

	Test	Standard 1	Standard 2	Standard 3
Deionized water	10ml	10ml	10ml	10ml
Serum	0.1ml	_	_	_
Standard 1	_	0.1ml	_	_
Standard 2	_	_	0.1ml	_
Standard 3	_	_	_	0.1ml

The tubes were well mixed then placed in the flame photometer. The air compressor was switched on and air pressure adjusted. Deionized water was introduced through the atomizer. The gas was turned on and adjusted to give fine sharp cones. Appropriate filters were then placed for simultaneous sodium and potassium estimation. Standard 1 (120/20) was introduced and adjusted 120 for sodium and 2.0 for potassium. The same was done with standard 2 and standard 3 to ensure that both displayed the exact concentration for both sodium and potassium. The diluted test serum was then introduced and the reading was taken and recorded.

ESTIMATION OF SERUM CHLORIDE CONCENTRATION

METHOD: Method of mercuric nitrate was used.

PRINCIPLE: This method involves titrating chloride ion with mercury ions, forming soluble but non-ionized mercury chloride. The end product is reached when excess Hg^{+} forms a complex with an indicator such as diphenylcarbazone producing a pale violet colour.

REAGENT: Mercuric nitrate, standard chloride prepared by dissolving 0.585g of NaCl in 50ml of distilled water. This gives a solution of 10Mmol/L and indicator prepared by dissolving 0.5g diphenylcarbazone in 50ml of 95% ethanol.

PROCEDURE

(a)Standardization of Mercuric Nitrate

To every 2ml of standard NaCl (10ml), 2 drops of indicator was added and titrated with mercuric nitrate. The end point was reached when the colour changed to pale violet. The procedure was repeated twice and the average of the three readings was taken.

(b) Titration of Serum

To 1.8ml of distilled water, 0.2ml of serum was added and titrated with mercuric nitrate. The end point was reached when colour changed from pale yellow to pale violet and the reading was taken.

STATISTICAL ANALYSIS

The data were expressed as mean ± standard deviation and analysed using statistical package for the social sciences (SPSS). Comparison was made between the test group and the control groups using the student's T-test and $p \leq 0.05$ was considered significance.

GRAPHICAL PRESENTATION OF RESULTS AND INTERPRETIONS

The results of the tested parameters are represented in the graphs bellow expressed as mean ± standard deviation

A represents Positive control group

B represents the Negative control group

C represents the test group treated with 250mg/kg plant extract while

D represents the test group treated with 500mg/kg plant extract.

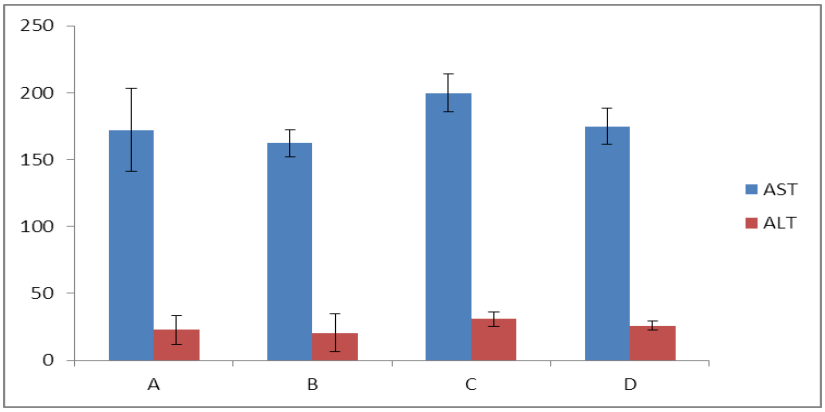

FIG 1 A graph comparing the levels of AST and ALT levels in TESTS GROUPS against the negative and positive control groups.

The level of AST in the test group C treated with 250mg/kg of plant extract increased non-significantly when compared to the positive control group A (p value>0.05) and increased significantly when compared to negative control group B ($p<0.05$) while the test group treated with 500mg/kg of plant extract non significantly increased when compared with the negative and positive control groups ($p>0.05$). The level of AST increased non significantly in both test groups when compared with the positive control group and negative control group ($p>0.05$), while ALT level non significantly increased in both test groups when compared with positive control group A and negative control group B

The level of MDA significantly increased in the test groups C and D when compared with the positive group A and negative group B ($p<0.05$) and the level of glutathione (GSH) significantly reduced in the test groups C and D, when compared with the positive and negative control groups A and B respectively ($p<0.05$)

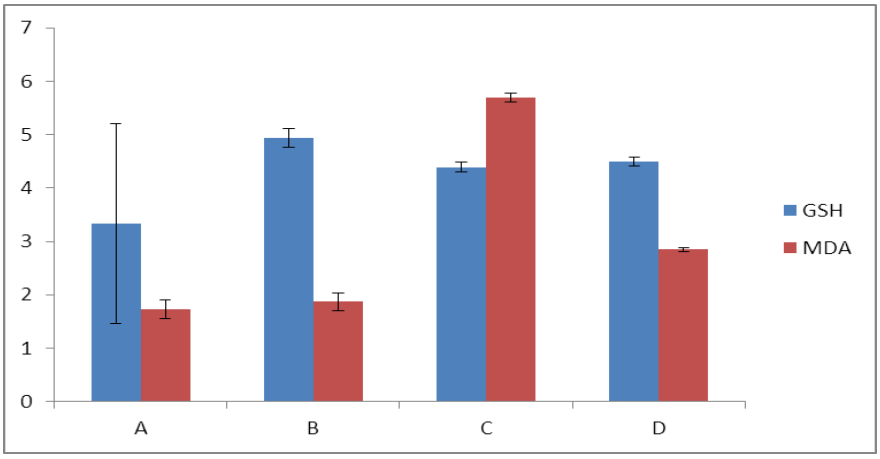

Fig.2 A graph comparing the levels of GSH and MDA levels in TESTS GROUPS against the negative and positive control groups.

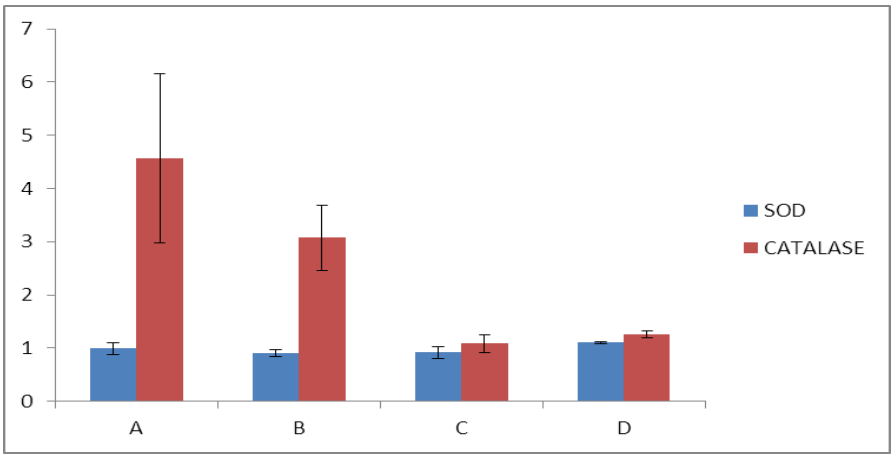

Fig3. A graph comparing the levels of SOD and CATALASE levels in tests groups against the negative and positive control groups.

The level of SOD non significantly reduced ($p>0.05$) in the test group C when compared to the positive control group A and non-significantly increased when compared with the negative control group B ($p>0.05$). It also reduces non-significantly in test group D when compared to the positive control group A ($p>0.05$) and significantly increased when compared to the negative control group B ($p<0.05$). Catalase activity decreased significantly in test groups C and D, when compared with the positive and negative group ($p<0.05$).

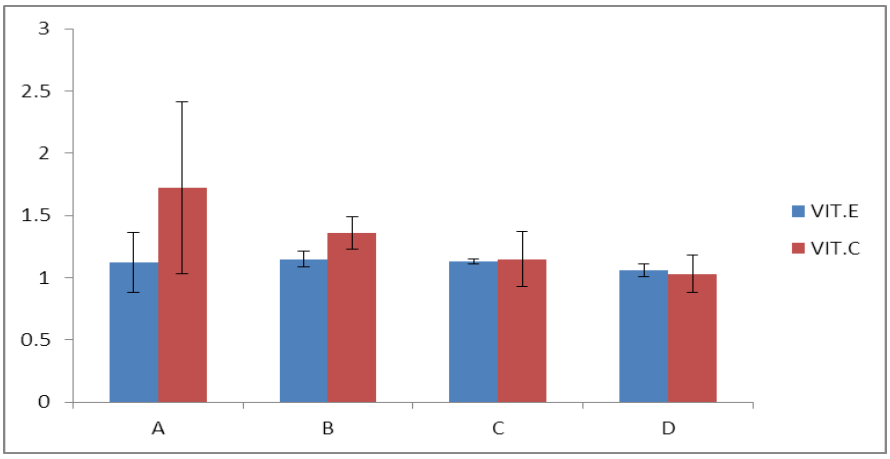

Fig.4. A graph comparing the levels of vitamin C and vitamin E levels in tests groups against the negative and positive control groups.

The level of vitamin E significantly reduced in test group C and D treated with 250mg/kg and 500mg/kg respectively when compared with the positive control group A (non-induced) and Group negative control group B (non-treated) ($p<0.05$) while vitamin C level non-significantly reduced in test group C when compared with positive control group A and negative control group B and significantly reduced when treated with 500mg/kg of plant extract ($p<0.05$) but showed a non-significant decrease when compared to positive control group A ($p>0.05$).

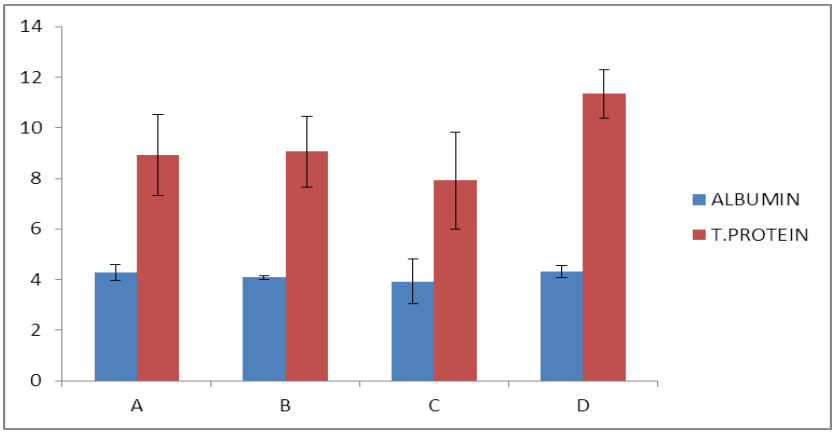

FIG 5. A graph comparing the levels of Albumin and Total protein levels in test groups against the negative and positive control groups.

The level of total protein non significantly reduced in test groups C when compared with positive control groups A and negative control group B ($p>0.05$), while test group treated with 500mg/kg of plant extract showed a non-significant increase ($p>0.05$). Also, albumin level non-significantly reduced in test group C when compared with the positive control group and negative control group ($p>0.05$), while test group D treated with 500mg/kg of

plant extract showed a non-significant increase when compared with negative control group B and positive control group A (p>0.05).

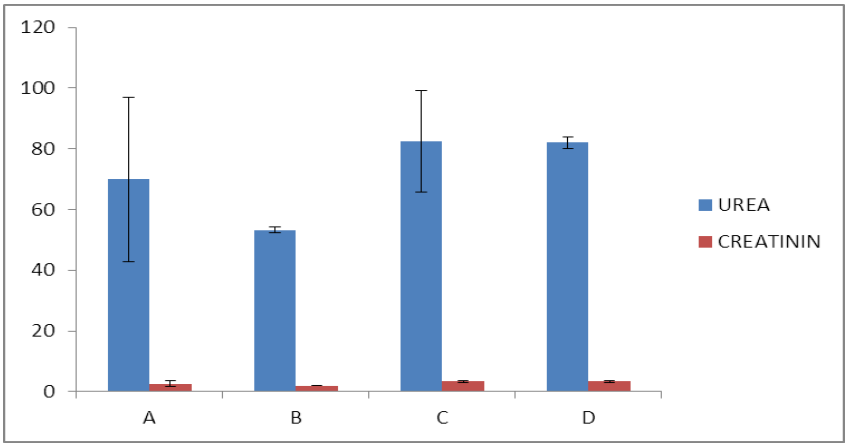

Fig 6. A graph comparing the levels of urea and Creatinin levels in test groups against the negative and positive control groups.

The graph showed a non-significant increase in urea concentration in test group C treated with 250mg/kg of plant extract when compared with the positive group A (p>0.05) and a significant increase when compared with the negative group B (p<0.05). Also, test group D, treated with 500mg/kg of plant extract showed a non-significant increase when compared with the positive control group A (p>0.05) and a significant increase when compared with the negative group B (p<0.05). Also creatinin level increased non- significantly in test group C when compared with the positive control group A (p>0.05) and significantly increased when compared with the negative control group B (p<0.05). Also test group D treated with 500mg/kg of plant extract showed a significant increase when compared with the negative group B (p<0.05) and showed a non-significant increase when compared with the non-induced group A (p>0.05).

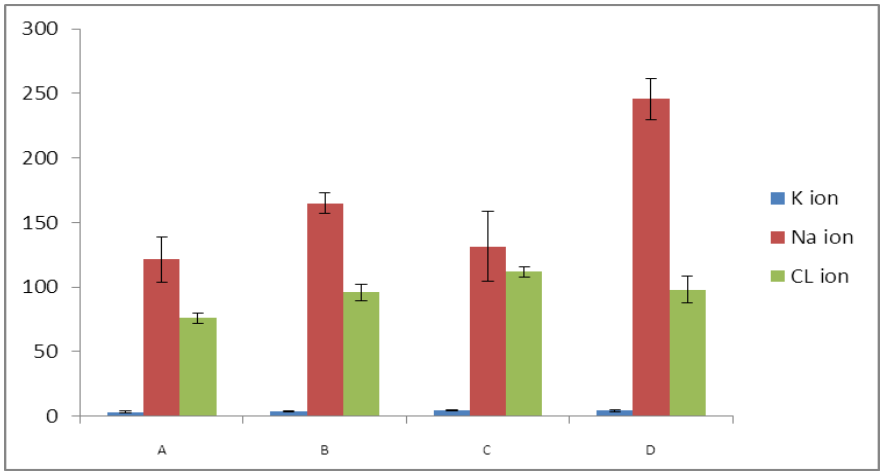

FIG 7. A graph comparing the levels of Na ion, K ion and Cl ion levels in test groups against the negative and positive control groups.

Sodium ion showed a significant increase in test group D treated with 500mg/kg of extract when compared to positive and negative control groups A and B respectively ($p<0.05$), while test group C treated with 250mg/kg showed a non- significant decrease when compared with the positive group ($p>0.05$) and a non-significant increase when compared with positive control group ($p>0..05$). Potassium ion non-significantly increased in both test groups C and D when compared with the positive control group and negative control group ($p>0.05$). Chloride ion showed a significant increase in test group C when compared with negative control group and positive groups. While test group D treated 500mg/kg of plant extract showed a significant increase when compared to the positive group ($p<0.05$) and a non-significant decrease when compared with the negative control group B ($p>0.05$).

DISCUSSION

Aspartate aminotransferase is an important enzyme marker that catalyses the reversible transfer of α- amino group between aspartate and glutamate and as such is an important enzyme in amino acid metabolism. They are found in the liver and other organs hence their use as a marker for liver injury/ damage which can lead to their leaking out in to the blood to an elevated level. Also Alanin aminotransrase is another marker enzyme resident in the liver, its elevated concentration in the blood is an indicator of liver damage alongside AST.

In this study, there was a non-significant increase in serum level of aspartate aminotransferase of the test groups treated with 250mg/kg and 500mg/kg of plant extract respectively when compared with the positive control group (the non-induced group). However, the AST level increased significantly in the test group receiving 250mg/kg plant extract when compared with the non-treated induced experimental animals. However alanine aminotransferase serum level was found to be non- significantly higher in the test group receiving 250mg/kg and 500mg/kg of plant extract. The non-significant increase in the AST/ALT level shows there was a liver damage but not severe as to cause elevated level in the blood, however, the increase in the AST level in the negative control group could be as result of other factors such as renal dysfunction or malfunctioning of other organs since AST aside being found in the liver are also found in other organs such as the kidney and the heart, implying that *Albuca nigritana plant* extract could be toxic in higher concentration. The toxicity could be as a result of the presence alkaloid which is known to be toxic at a high concentration, according to the work of (Edeoga *et al.,* 2007). The non-significant increase

suggests the plant as having no effect at reducing AST/ALT level in the in case of liver injury.

The liver plays a very important role in the synthesis of amino acids which is the building block of proteins. Proteins are important as building block of all cells and tissues. The body contain two types of protein; Globulin and albumin. Albumin helps to transport other substances within the body and also play an important role in preventing the fluids of the body from leaking out, while globulin include enzymes, antibodies and more than 500 other different proteins. During liver injury, the level of total protein inceases (O'Connell, 2005), this is because during tissue inflammation, the immune system continuously sends out antibodies which are proteins that try to attack the diseased cell and prevent them from causing further harm. As a result the level of protein continues to rise. In this study, the level of total proteins in test animals induced with liver damage was higher when compared with the non- induced control group. However those treated with plant extract of 250mg/kg and 500mg/kg respectively showed a non-significant decrease in serum total protein level when compared with the non- treated induced experimental animals implying that the plant extract cannot be used in hepatocellular protection. The non-significant decrease in the test group when compared to the non-induced group shows that the plant extract lack the potential of reversing the effect of low serum total protein which could also occur as a result of liver damage of dysfunction.

Albumin is a type of protein made specifically by the liver; it is the main constituent of total protein. Low level can occur in liver damage/ disease, nephrotic syndrome, burns, protein losing entropathy, malabsorbtion and genetic variations. High level can occur as a result of dehydration (Gaull et *al.*, 1984).

In this present study, there was a decrease in albumin level in the non-treated induced hepato-cellular experimental animals when compared with the non- induced experimental animal indicating liver damage. Treatment with 250mg/kg per body weight of the plant extract showed a non-significant decrease in the albumin level when compared with the negative control experimental animals and the non-induced experimental implying the non-protective effect of the plant extract on the hepatocyte. Treatment with 500mg/kg of plant extract showed a non-significant increase in albumin level when compared to both the non-treated induced rats and the non-induced rats, also implying that the plant extract lack the potential

of reversing the effect of the liver damage which is evident in the decrease concentration of albumin in non- treated induced experimental an animals.

Urea is a by-product of protein breakdown which starts from the liver. The liver produces urea as a waste product of digestion of protein (Lingo, 2008). Urea level could increase or decrease depending on the organ that is being affected. High urea blood urea nitrogen is an indication of renal failure, low glomerular filtration rate, congestive heart failure and gastrointestinal haemorrhage, while low serum urea nitrogen occur in the case of severe liver injury/disease(Lingo, 2008). In this present study in which the level serum urea concentration was investigated showed a reduction in serum urea concentration in experimental animals induced with liver damage without treatment when compared with the positive control group. Treatment with 250mg/kg on test group showed a non-significant increase when compared with the positive control groups and a significant increase when compared with negative control groups. Treatment with 500mg 0f plant extract on the test group D also showed a non- significant increase when compared with the positive control groups and a significant increase when compared with the negative control group. The significant increase in the urea serum level in the test group maybe as result of a positive effect the plant extract has on the liver which help to reverse the dysfunction in the protein breakdown or maybe due to adverse effect on the kidney which hinder to adequate excretion of the urea from the body.

Creatinine is a chemical waste product that is generated from muscle metabolism. Creatinine is produced from creatin an important amino acid synthesized in the liver and transported to the muscle for metabolism. Creatinine is transported through the bloodstream to the kidneys where they are filtered out and disposed of in the urine (Allen, 2012) Creatinine are not normally found in higher than normal concentration in the blood as the kidney helps to maintain creatinine level at normal range, hence creatinine level has been reliably used as an indicator of kidney function. High level of creatinine occurs as a result of impaired kidney function while low level occurs as a result of loss of liver function in creatin synthesis (Taylor, 1989).

In this study, creatinine level significantly increased in the test group treated with 250mg/kg and 500mg/kg of plant extract when compared with the non-treated induced control group. This implies impairment in the kidney function which may be caused by the chloroform/hexane used to induce liver damage whose effect could not be reversed by the plant extract.

Malondialdehye result from lipid peroxidation of poly unsaturated fatty acid (Davey *et al*, 2005). Lipid peroxidation refers to the oxidative degradation of lipid which results in release of free radicals resulting in cell membrane damage. Lipid peroxidation has been associated with liver damage following the work of Parola *et al,* 1996. In this study the level MDA was investigated to determine the level of hydrogen peroxidation which is an indicator for liver damage. There was an increase in the level of MDA when negative control group was compared with the positive control group indicating lipid peroxidation which is an evidence of liver dysfunction. On treatment with 250mg/kg of plant extract, the test group showed a significant increase in MDA when compared with the positive group and with the positive control group in. It also showed a significant increase at a higher dose of 500mg/kg of plant extract when the test group were compared with the negative control group and the positive control group. The increase implies an increase in level of lipid peroxidation which is an evidence of liver damage which could not be reversed by treatment with plant extract, hence the plant extract lacks the potential of reversing liver damage and cannot be said to be protective.

Glutathion is an important antioxidant by the body to scavenge free radicals that may occur in tissue damage. The level of glutathione was investigated in this study to ascertain the level of antioxidant activity in chloroform/hexane induced liver damage and the level of antioxidant activity of this antioxidant showed a non-significant increase when the test group that received 250mg/kg of plant extract were used to compare with the positive control group and significantly reduced when the test group were compared with the negative control group. The decrease that occurred when compared with the negative group showed a high level of free radical activity in liver damage which the plant extract could not reverse hence the plant extract lack glutathione activity.

Superoxide dismutase is a class of antioxidant that catalyse the breakdown of the break-down of superoxide anion into oxygen and hydrogen peroxide. SOD activity was investigated in this study and found to be dose dependent as it had a significant increase in the test group treated with 500mg/kg of plant extract when compared with the negative control group and non- significant increase when compared with the positive control group, while the test group treated with 250mg/kg showed a non- significant decrease when compared with the positive control group and a non -significant and the test group treated with 500mg/kg showed a non- significant decrease when compared with the positive control group. This suggests that

at a higher dose, *Albuca nigritana* plant extract will have effect at increasing serum SOD level.

Catalase is a very important enzyme in protecting cell from oxidative damage by reacting with oxidative species (Boon *et al*, 2007). Its decrease in the blood in the condition of damaged liver is an indicator of increase activity of free radicals which causes it continuous use up much more than is being produced. In the study under consideration, catalase activity significantly reduced in the test groups treated with 250mg/kg and 500mg/kg when compared to the negative control and positive control groups respectively. This indicates an increase in free radical production which is as a result of the Chloroform /hexane induced hepatocellular damage which the plant extract was not able to reverse and hence the continuous increase in catalase use up leading to its significant decrease. Therefore it can be said that the plant extract cannot be used to increase catalase activity.

Vitamin C is a monosaccharide oxidation-reduction catalyst found in both plant and animal. They also serve as a cofactor in most of the enzymatic reactions which facilitate wound healing and prevent bleeding from capillary. Vitamin C also serves as an antioxidant against oxidative stress (Padayatty *et al*, 2003). Vitamin C are synthesised internally in the body of almost every organism. Its activity is reduced in excess production of free radicals. In this study, the test group treated with 250mg/kg of plant extract showed a non- significant decrease when compared to the positive control group and negative control group, while those treated with 500mg/kg of plant extract showed a significant decrease when compared to the negative control group, and a non-significant decrease when compared to the positive control group. This shows that the plant extract lack the potential of increasing Vitamin C activity which was reduced probably due to the induced liver damage that was not reversed by the plant extract at two different doses.

Vitamin E is fat soluble vitamins that act as antioxidant against lipid peroxidation (Herera and Barbas, 2001). The level of vitamin E activity was investigated in this study against lipid peroxidation which occur in liver damage and was found to increase non-significantly in the test group treated 250mg/kg of plant extract when compared to negative control group and positive control groups, also at 500mg/kg concentration of the plant extract, it also showed a non- significant decrease when compared to the positive control group animals and a significant decrease when compared to the negative control group. This suggests that the

plant extract does not have the potential of increasing Vitamin E activity hence cannot be said to have a hepatocellular protective potential.

Sodium is the major cation found extracellular fluid. Sodium regulates the total amount of water ion the body. Increase serum sodium level occurs whenever there is excess sodium in relation to water. This normally occur in kidney dys- function or kidney disease and low serum sodium level occur whenever there is a little increase in the amount of body water relative to sodium. This happens with the disease of the liver and the kidney. In this study, the amount of sodium ion in blood serum of haloalkane induced hepatocellular damage was investigated. There was a relative increase in the level of sodium ion in the blood serum of the non- treated experimental animals when compared with the non- induced group. The relative increase suggests a renal dysfunction which might have inhibited the excretion of sodium ion through the urine. Test group treated with 250mg/kg of plant extract showed a non- significant increase when compared with the positive control group and a non-significant decrease when compared with the negative control group. Furthermore, test group treated with 500mg/kg of plant extract showed a significant increase in the sodium ion concentration when compared with the positive control group and negative control groups respectively, showing that the sodium concentration was not reduced. Hence, it can be said that the plant extract lacks the potential in reversing the disorder that might have occurred in the kidney resulting in the rentation of sodium ion the blood.

Potassium is the major cation found intracellular fluid. Potassium is normally excreted by the kidneys; therefore disorders that decrease the function of the kidneys can result in result in high protein level. In this study, the level of potassium increased when non- treated induced experimental animals were compared with the normal group indicating a potential kidney disorder. The study conducted using the test groups treated with 250mg/kg and 500mg/kg of plant extract showed a non- significant increase in potassium concentration when compared to the positive control group and the negative control group, indicating that the plant extract has no effect at reducing high potassium concentration in the case of kidney disorder.

Chloride ion is the major anion found in the body that made up the extracellular fluid. Chloride ion plays a role in helping the body to maintain a normal balance of fluid. Increase in chloride ion may be seen in certain kidney diseases and sometimes in the over-activity of parathyroid glands. In the present study the chloride ion level was investigated in test group of experimental animals treated with 250mg/kg of plant extract and 500mg/kg of plant

extract. In the experimental animals induced with hepatocellular damage without treatment showed a relative increase in chloride ion when compared with the non- induced. The test group treated with 250mg/kg plant extract showed a significant increase in chloride ion concentration when compared with the positive control and negative control group, while those treated with 500mg/kg plant extract showed a significant increase when compared with the positive control group and a non -significant increase when compared with the negative control group. This showed a high level of chloride ion the blood serum which is an indicator of kidney dysfunction which the plants extract could not reverse. Hence it can be said that extract lack renal function potential.

CONCLUSION

The study was carried out to investigate the hepatoprotective effect of methanol extract of *Albuca nigritana* bulb on hexane/chloroform induced hepatocellular damage on female wistar rats. The plant extract showed non reducing effect on the albumin, urea, creatinine and malondialdehyde serum concentration as they were non-significantly reduced ($p>0.05$) in the test groups. Also there was a significant increase in AST at 250mg/kg body weight concentration even though the ALT level increased non-significantly. The test on antioxidant activity showed a significant decrease in catalase, glutathione, superoxide dismutase, vitamin E and vitamin C activity and on the test for electrolyte activity, sodium ion and chloride ion increased significantly, while potassium ion increase non-significantly. Based on the various negative effects the plant extract has on the greater percentage of the various enzyme markers and biochemical paramerters tested for, conclusion can be made that *Albuca nigritana* bulb extract has no protective effect on the experimental animal and renal function.

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