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EFFECTS OF ETHANOLIC EXTRACT OF *CYPERUS ESCULENTUS* (TIGER NUT) ON SOME LIVER FUNCTION PARAMETERS USING ALBINO WISTAR RATS

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ABSTRACT

This study investigates the effects of ethanolic extract of *Cyperus esculentus* on some liver function parameters. Thirty six albino wistar rats weighing between 180g and 220g were randomly grouped into three (two test groups and a control group) of 12 rats each. After two weeks of acclimatization, Groups 2 and 3 (test groups) were orally administered with 800mg/kg and 400mg/kg of the ethanolic extract of *Cyperus esculentus* respectively for twenty eight days while group 1 (control group). Were allowed asses to water and feed. After every seven days of extract administration, three rats from each group were sacrificed and blood samples were collected by cardiac puncture for biochemical analysis of some liver enzymes (alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), serum total protein and serum albumin). Livers of the sacrificed animals were also harvested for histological studies. Data obtained from the study were analyzed statistically. Result showed a dose dependent significant ($P<0.05$) increase in serum concentration of ALT and ALP. Serum concentration of total protein, albumin and AST were not overtly affected. The histological study of the harvested liver showed hepatocytes degeneration and periportal inflammations indicating alteration in the normal physiological status of the liver. Findings from this study showed that the extract could be unhealthy to the liver; hence care should be taken when consuming the fruit.

KEYWORDS: *Cyperus esculentus,* liver enzymes, hepatocytes and inflammation.

INTRODUCTION

Plant derived products have been used for medicinal and nutritive purposes for centuries,[1] This usage has gained prominence worldwide over the last three decades. An estimated 80% of the population in developing countries relies on plant preparation as medicines to take care of their health needs.[2] This surge in the use of plant products as medicine is believed to be due to their accessibility, affordability and the perceived failure of synthetic drugs in the treatment of some chronic diseases like hypertension, diabetes, arteriosclerosis, etc.[3] According World Health Organization (WHO), about 65-80% of the world's population especially in developing countries depends on plants and their formulations for their primary health care due to poverty and lack of access to modern medicine.[4]

Medicinal plants and their formulations have continued to attract attention as a result of the strong speculations and belief that they are safe and very effective,[5] This strong speculation and assumption has led to its indiscriminate use especially in developing countries.[6] Recently, some studies show that some of these medicinal plants alter the physiological status of some vital organs in the body while effecting their medicinal actions,[6] Consequently, it has become imperative to ascertain the effects of those plants used as medicines on the physiological status of vital organs. This study therefore investigates the effect of "*Cyperus esculentus"* one one the widely eaten nuts in Africa especially in Nigeria on the physiological status of the liver. The liver is our target organ because among other functions, it takes up numerous toxic compounds and drugs which may include medicinal plant formulations from the portal circulation.[7] and detoxifies them. Therefore, toxicity of substances are determined by how much it affects the activity of the liver which is marked by measuring the plasma concentration of some biochemical compounds and enzymes produced by the liver (liver makers). Such liver makers include total serum protein, albumin, triglycerides, and total cholesterol, high and low density lipoproteins, and liver enzymes like alkaline phosphatase, aspartate transaminase and alanine transaminase.

Cyperus esculentus

Cyperus esculentus is a grass like plant belonging to the cyperaceae family,[8,9] It is native to warm temperate of subtropical regions of the northern hemisphere.[10] It is cultivated today in China, Spain and West African countries like Niger, Mali, Senegal, Ghana, Togo and Northern Nigeria,[11,12] *Cyperus esculentus* is sweet nut like tubers known as Tiger nut.[13] This nut is known by other names like earth almond, rush nut, chufa (in spanish) yellow nut,

edible galingale, edible rush etc.[14,15,16] In Nigeria the Hausas call it aya, the Igbos call it ofio, and in southern Nigeria it is commonly called aki hausa or hausa groungnut.[17] Tiger nut can be eaten raw, roasted, dried, baked or it can be made into a refreshing beverage called (Hochata De Chufas) or tiger nut milk.[18] Also, it can be used as a flavoring agent for ice cream and Biscuits.[19] Phytochemically, *Cyperus esculentus* has been reported to contain alkaloids, sterols, resins, cyanogenic glycosides, saponins and tannins.[20] The nut is rich in energy content (starch, fat, sugars and protein), minerals (phosphorus, potassium) and vitamins E and C.[21] Medicinally, Tiger nut has been reported to be effective in the prevention of heart attack, thrombosis and activates blood circulation. It has been reported to reduce the risk of colon cancer,[22] and is suitable for diabetic patients.[23] It has equally been reported to have high content of oleic acid with positive effect on lowering cholesterol level due to its high content of vitamin E. The nut has been found to be ideal for children, older persons and sportsmen,[24] The tubers contain about 25 % oil, which are resistant to peroxidation, 50 % digestible carbohydrates, 4 % protein and 9 % crude fiber.[25,26] Oil extracted from tiger nut can be used as food oil as well as industrial purposes,[27,28] The very high fibre content combined with a delicious taste; make them ideal for healthy eating.[29]

MATERIALS AND METHODS

Experimental Animals

Thirty six (36) male albino Wistar rats weighing (180 – 220g) were purchased and acclimatized at the animal house, Department of Human Physiology, College of Health Sciences, University of Port Harcourt, Rivers State, Nigeria. The animals were kept in a well ventilated cage at room temperature of 12 hours light and dark cycle and acclimatize for 14 days. They were allowed free access to feed diet (Top Feeds, Broiler finisher – Product of Eastern premier feed mills Ltd.) and water *ad libitum*.

All animal experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals (Pub No. 85-23, Revised 1985).

Collection and Identification of Plant Materials

Dried tubers of *C. esculentus* (Tiger nuts) were purchased from Katokuo Area of Kano State Nigeria. They were correctly identified and authenticated in the Herbarium Unit of the Department of Plant Science and Biotechnology, University of Port Harcourt.

Preparation of Ethanolic Extracts of *Cyperus esculentus*

Following established method.[30] tubers of *Cyperus esculentus* (tiger nuts) were rid of bad tubers and dirt, and washed clean. Thereafter, the tubers were milled to fine powder using manual engine grinder (Modelcorene, A.5 lander YCIA S.A). The milled sample was soaked in 5L of 80% Ethanol for 48hours. Thereafter, it was filtered with Whatman No. 1 filter paper to separate the filtrate from the residue. The filtrate was then concentrated under reduced pressure in a vacuum at 45^{o}C using a rotary evaporator (Searl Instruments Ltd. England) into a colloid form and stored at 4^{o}c.

Experimental Design

Thirty six albino wistar rats weighing between 180 -220g used for this study were randomized into 3 groups of 12 rats each. Group 1 Served as the control and were allowed free access to feed and water. Group 2 were orally administered with 800mg/kg of the extract while Group 3 was also orally administered with 400mg/kg of the extract. The choice of dosage was informed by reported safe dose of up to 2000mg/kg.[20] The extracts were administered orally in the early hours of each day (within 8am to 10am) throughout the period of four weeks administration.

Three rats from each group were sacrificed after every seven days of daily administration of the extract (that is, on day 8, day 15, day 22 and day 29). Blood samples were collected through cardiac puncture into lithium heparin bottles for biochemical analysis and the liver of each rat was collected via abdomino-thoracic dissection into plain bottle containing buffered formalin for histological study.

Analysis of of experimental Parameters

The collected blood samples were centrifuged at 5000rpm for 10 minutes to obtain clear serum for the biochemical analysis. The serum supernatant was then carefully aspirated with needled syringe and stored in a plain sample bottle. The biochemical analysis for serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase (ALP), total serum protein and serum albumin were done using Mindray Auto-analyzer machine (Model: BS – 800M) at the of the Department of Chemical Pathology, University of Port Harcourt Teaching Hospital following standard laboratory procedures. Also, standard histopathological technique.[31] was followed in the histopathological examination of the liver.

Statistical Analysis

At the end of the study, data obtained were analyzed using Statistical Package for Social Sciences (SPSS Version 20.0). One-Way Analysis of Variance (ANOVA) was used to compare the means and Dunnett's post-test Comparison was used to test for statistically significant differences between control and test groups with a statistically significant difference at $P< 0.05$.

RESULTS AND DISCURSION

Results from the biochemical analysis and histological study are presented in figures 2-11 below.

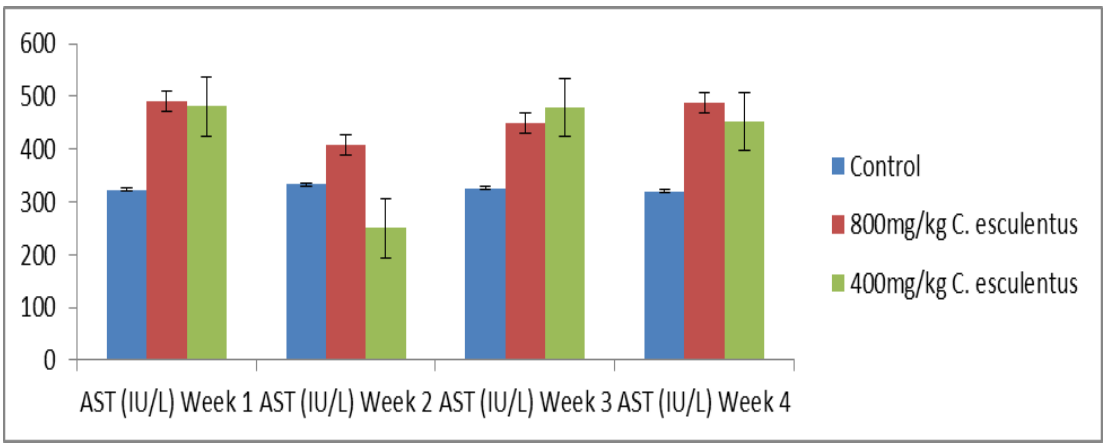

Figure 2: Effects of ethanolic extract of *C. esculentus* on serum AST (IU/L)

The result of serum AST concentrations of rats administered 800mg/kg and 400mg/kg doses of the ethanolic extract of *C. esculentus* is presented in the figure above. The result showed a non significant ($P>0.05$) difference when compared with that of the control group.

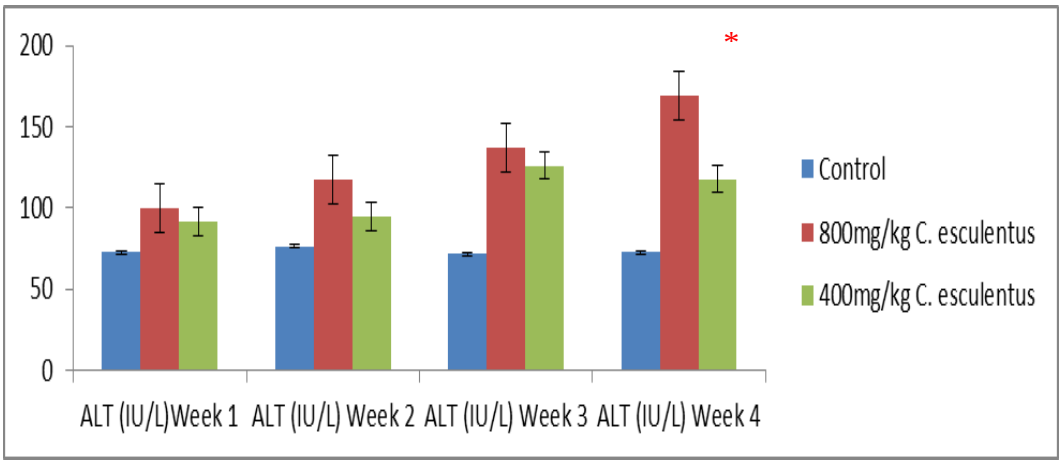

Figure 3: Effects of ethanolic extract of *C. esculentus* on serum ALT (IU/L)

The result of serum ALT concentrations of rats administered 800mg/kg and 400mg/kg doses of the extract is presented in the figure above. The result showed a dose and duration dependent significant (P<0.05) increase when compared with that of the control group.

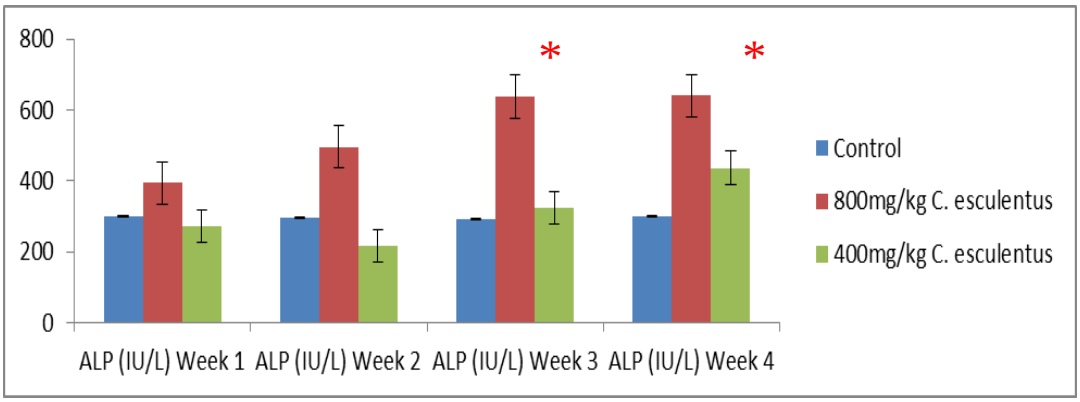

Figure 4: Effects of ethanolic extract of *C. esculentus* on serum ALP (IU/L)

The effects of 800mg/kg and 400mg/kg doses of the extract on serum ALP of rats is presented in the figure above. The result showed a dose and duration dependent significant (P>0.05) increase in the test groups when compared with that of the control group.

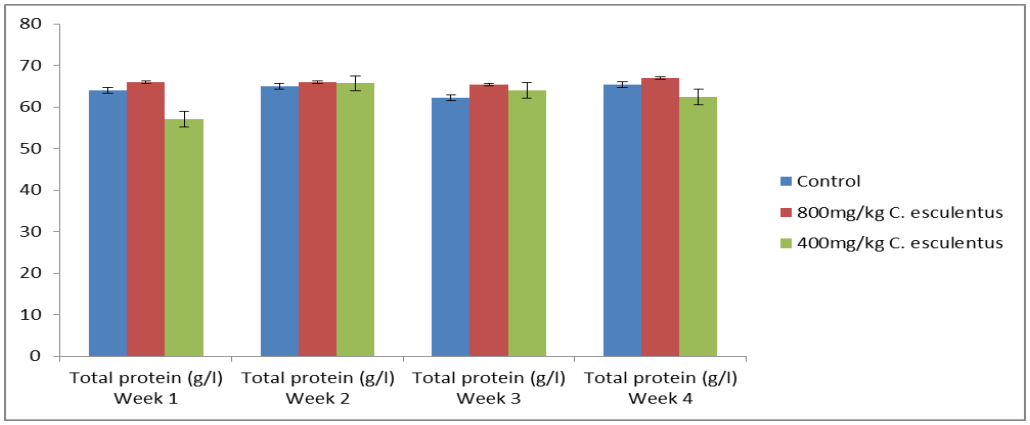

Figure 5: Effects of ethanolic extract of *C. esculentus* on total serum protein (g/l)

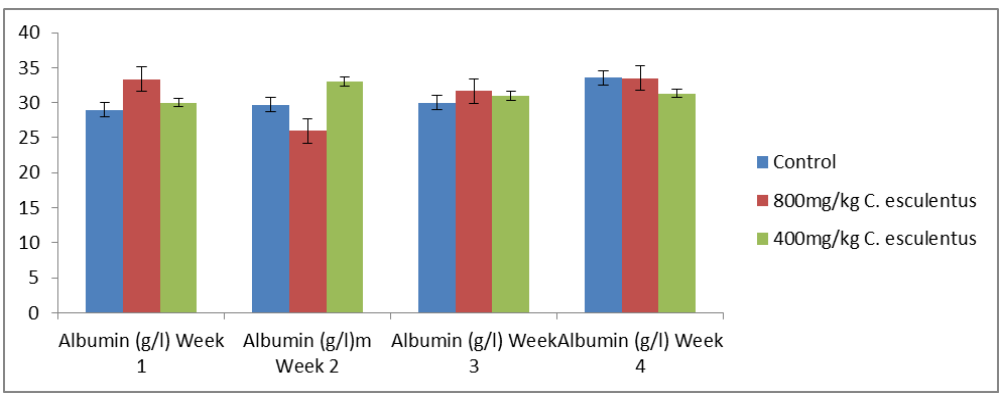

Figure 6: Effects of ethanolic extract of *C. esculentus* on serum albumin (g/l)

The results of serum total protein and serum albumin of rats administered 800mg/kg and 400mg/kg doses of the extract are presented on figures 5 and 6 respectively. The results showed non significant effect on either of the parameters.

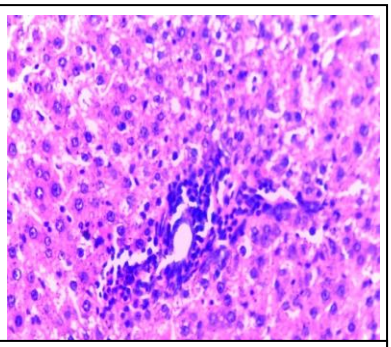

Fig. 8: Effects of one week administration of 800mg/kg *C. esculentus* on histology of liver: hepatocytes & Periportal inflammation. (mag. X200)

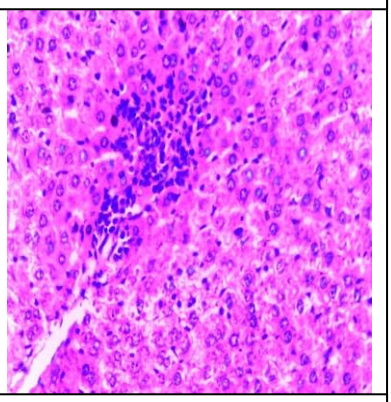

Fig. 9: Effects of one week administration of 400mg/kg *C. esculentus* on histology of liver: Hepatocytes infla- mamation. (mag. X200)

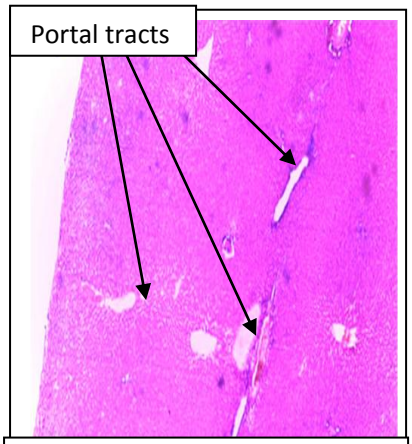

Fig. 7: Histology of normal liver (control rat)

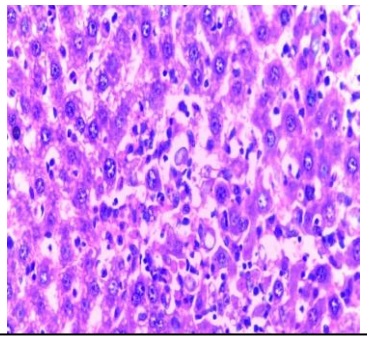

Fig. 10: Effects of four weeks administration of 800mg/kg *C. esculentus* on histology of liver: Inflammation & mild fatty change. (mag. X200)

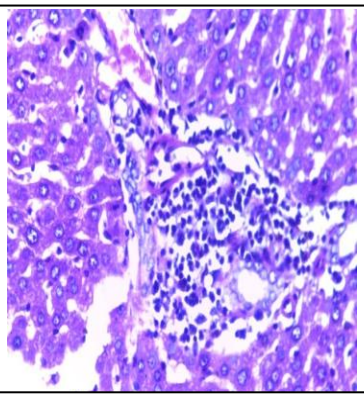

Fig. 11: Effects of four weeks adminis-tration of 400mg/kg *C. esculentus* on histology of liver: Periportal & vacuolar degeneration. (mag. X200)

DISCUSSION

The physiological status of the liver is mainly determined by measuring the plasma levels of some liver enzymes which include alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT).[32] also, other biochemicals produced by the liver which include total serum protein, albumin, bilirubin, triglycerides, total cholesterol, high and low density lipoproteins can be assayed. Normally, ALT and AST are in high concentrations within the liver cells (hepatocytes). Damage or destruction of the hepatocytes leads to the release of these enzymes into circulation thereby increasing their plasma concentration. Thus, increase in the plasma or serum level of any of these enzymes is an indication of hepatocytes damage.[33] From the results of this study, ethanolic extract of *Cyperus esculentus* at

400mg/kg and 800mg/kg did not cause any significant (P>0.05) increase in the serum concentration of AST (fig. 2). However, a significant (P>0.05) increase in the serum concentration of ALT was observed at a dosage of 800mg/kg of the extract after the fourth week of administration. This is seen in figure 3. Moreover, the dose and time dependent significant (P>0.05) increase in serum concentration of ALP seen in this study could be as a result of intrahepatic cholestasis or infiltrative diseases caused by some components of the extract.(figure 4). The various degrees of periportal inflammation and hepatocytes degenerations seen in the photomicrograph of the harvested livers (fig. 8 - 11) explains the observed significant (P>0.05) increases in the serum concentrations of the assayed enzymes. Therefore *Cyperus esculentus* tubers (tiger nuts) when frequently and consistently consumed could gradually destroy liver cells and threaten life.

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