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RESEARCH PAPER

THE EFFECT OF AQUEOUS EXTRACT OF *CYPERUS ESCULENTUS* ON SOME LIVER FUNCTIONAL INDICES IN RABBIT

*¹Ebojele F.O. and ¹Ezenwanne E.B.

Department of Physiology, School of Basic Medical Sciences, College of Medical sciences, University of Benin, Benin city, Nigeria.

*Corresponding author: fredojele78@yahoo.com

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ABSTRACT

Twenty-five (25) adult female rabbits were employed to study the possible effects of aqueous extract of *Cyperus esculentus* on some liver functional indices. The rabbits were divided into five groups (A, B, C, D and E) of five animals each with group A serving as control. The experimental groups (B, C, D and E) received daily oral doses of 12.5mg, 25mg, 50mg and 100mg per kg of body weight of the extract respectively for a period of four weeks. Weekly estimate of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were carried out. Statistical analysis was done using ANOVA and the results were expressed as Mean \pm SEM, with p-values of less than 0.05 considered statistically significant. The results revealed significant increase in serum ALT, AST, and ALP concentrations in group E at the end of the experiment. It was concluded that the observed increase in serum levels of these enzymes indicate that *Cyperus esculentus* may have some clearly definable hepatotoxic properties, probably, if taken in large quantities and for a prolonged period.

Keywords: *Cyperus esculentus*, Liver, Rabbit, Aqueous extract, Enzymes.

INTRODUCTION

Cyperus esculentus, also known as 'Tiger nut' in local parlance, is a plant of the family Cyperaceae. The plant produces rhizomes from the base and tubers that are somewhat spherical (Cortes *et al.*, 2005), and is also known by various names like yellow nutsedge, Chufa (a Spanish name), Earth Almond, Rush nut, Flatsedge, Water grass, Zulu nut, Nut grass, and edible rush (Shilenko *et al.*, 1979; Eteshola and Oraedu, 1996). The nut of this plant is known by various local languages in Nigeria as 'Aya' in Hausa, 'Imumu' in Yoruba, and 'Ofio' or 'Aki Hausa' in Ibo (Omode *et al.*, 1995). The nuts can be eaten raw, roasted, dried, baked or made into a refreshing beverage called 'kunnu' in Hausa language (Oladele and Aina, 2007).

Cyperus esculentus is valued for the highly nutritional starch content, dietary fibre and digestible carbohydrate of monosaccharides, disaccharides and polysaccharides (Temple *et al.*, 1990). The nut was also reported to be rich in sucrose, protein, minerals and fat, which are resistant to peroxidation (Rita, 2009). It was also reported that *Cyperus esculentus* can be taken by diabetics mainly for its sucrose and starch, and for its high content of arginine which is reported to stimulate the production of insulin (Belewu and Belewu, 2007). The plant was shown to be nutritious, and possesses anti-sickling activity and may therefore be helpful in the management of sickle cell patients (Monago and Uwakwe, 2009). One other study showed that *Cyperus esculentus* content of arginine (an antisickling amino acid) is very high (Nwaogukpe and Nwazue, 2010). This fact is potentially of immense benefit as an antisickling agent in inhibiting sickle cell hemoglobin polymerization, improvement of the oxidant status of sickle erythrocytes and improvement of the oxygen affinity. Thus, *Cyperus esculentus* is probably of profound nutritional value as it possesses antisickling properties for the effective treatment and management of anemia, kwashiorkor, thalassemia,

sickle cell disease and their complications (Nwaoguikpe and Nwazue, 2010). These and several other reports led some group of workers to understudy the phytochemical composition of *Cyperus esculentus* tuber, following which it was determined that it contains Alkaloids, Cyanogenic glycosides, Resins, Tannins, Sterols, and Saponins (Ekeanyanwu *et al.*, 2010).

There was a study of the possible effect of *Cyperus esculentus* on heart diseases and related conditions. In their report, the nuts were found to be ideal for children, the elderly, and sportsmen and women as a result of its cholesterol lowering properties (Martinez, 2003). There is a report that the consumption of the oil from *Cyperus esculentus* tuber decreases the risk of cardiovascular diseases because of the low content of saturated fatty acid (Burn *et al.*, 2004). It was also reported to help in preventing thrombosis, and the tuber was also implicated in the reduction of colon cancer (Adejuyitan *et al.*, 2009). There is paucity of information from literature on the effect of *Cyperus esculentus* on liver function. It was therefore the aim of the present study to determine the effect of aqueous extract of *Cyperus esculentus* on some liver functional indices in rabbit.

MATERIALS AND METHODS

Experimental animals: Twenty-five female rabbits of comparable age with initial mean weight of between 0.9kg and 1.6kg were procured from the animal house of the Faculty of Agriculture, University of Benin. The animals were thereafter transferred to the animal house of the Department of Pharmacology, University of Benin, where the experiments were carried out.

The animals were kept in a stainless steel cage with wire mesh floor and allowed to acclimatize for a period of two weeks on normal feeds and water before the commencement of the experiments. Animal management and experimental protocols were carried out in accordance with the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Guide, 1985).

Substance of study: Fresh *Cyperus esculentus* nuts were purchased from a local market in Benin City, Edo State, Nigeria, and authenticated in the Department of Plant Biology and Biotechnology, University of Benin.

Substance preparation: The nuts were washed and oven dried at 37°C for 24hrs and thereafter, pulverized into smooth powder using an impact mill. The pulverized nut (850g) was mixed with 6 litres of distilled water and left for 24hrs. The mixture was stirred at 3hrs interval using a sterile rod and passed through sieving cloth. The filtrate was concentrated over a water bath and yielded 52.94%. The dried extract was stored at 4°C prior to use.

Animal grouping: The animals were placed into five (5) groups (A, B, C, D and E) made up of five (5) rabbits per group. Group A served as control while groups B, C, D and E served as the treatment groups.

Substance administration: The treatment groups received daily oral dose of 12.5mg, 25mg, 50mg, and 100mg per kg of body weight of the extract respectively for a period of four weeks while the control group received an equal volume of distilled water. Administration of the extract was done orally, using orogastric tube to ensure that the animals in each group received equal amount of the extract.

Sample collection: The baseline values of serum concentrations of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and body weight were determined in all groups.

At the end of each week, blood samples were collected from the marginal ear vein using hypodermic syringes and needles as described by McClure (1999). The blood samples were stored in sample containers and later taken to the laboratory for analyses of serum ALT, AST, and ALP.

Sample analysis: Serum ALT and AST activities for cellular liver integrity were estimated with the Randox reagent kit using 2,4-dinitrophenylhydrazine substrate as described by Reitman and Frankel (1957). ALP activity for biliary tract integrity was determined with the Randox reagent kit using the p-nitrophenylphosphate substrate as described by Bassey *et al.*, (1946).

Data analysis: The SPSS version 16.0 was used for the data analysis. Comparison between groups was done using ANOVA and post hoc test was done with Least Significant Difference (LSD). The results were presented as Mean \pm SEM (Standard Error of Mean) and P-values of less than 0.05 considered statistically significant.

RESULTS

The serum concentration of ALT (Table 1) increased significantly ($p<0.05$; $p<0.005$; $p<0.001$) mostly in animals of group E during the second, third and fourth weeks, compared to the control. Similarly, the enzyme AST also showed significant increase ($p<0.05$; $p<0.005$) in serum level in the animals of group E during the second, third, and fourth weeks (Table 2). The changes in serum ALP concentration is presented in Table 3. A significant increase ($p<0.05$; $p<0.005$; $p<0.001$) was observed in all the treated groups during the second to fourth week. This increase was also observed among the control. There was observable (but not statistically significant) increase in the weight of the animals (Table 4).

Table 1: Mean serum alanine transaminase(ALT) concentration (u/l) in Rabbits treated with aqueous extract of *Cyperus esculentus* for a period of four weeks.

	Group A (control)	Group B (12.5mg/kg)	Group C (25mg/kg)	Group D (50mg/kg)	Group E (100mg/kg)
Baseline	18.20±0.58	20.20±0.73	18.00±0.55	18.20±1.77	19.80±0.80
Week 1	18.80±1.39	20.40±1.03	18.80±0.49	19.40±0.75	20.40±0.98
Week 2	20.00±1.41	22.40±1.36	19.80±2.13	21.60±1.60	29.40±1.21***
Week 3	19.00±1.52	21.40±1.29	18.40±1.44	21.00±2.02	26.40±1.29**
Week 4	19.80±1.71	22.80±1.59	19.80±2.24	21.40±2.29	27.60±0.68*

Significant values are Mean ± SEM compared to control (* $P<0.05$, ** $P<0.005$, *** $P<0.001$) n=5

Table 2: Mean serum aspartate transaminase(AST) concentration (u/l) in Rabbits treated with aqueous extract of *Cyperus esculentus* for a period of four weeks.

	Group A (control)	Group B (12.5mg/kg)	Group C (25mg/kg)	Group D (50mg/kg)	Group E (100mg/kg)
Baseline	18.80±0.20	20.40±1.08	14.40±1.17*	14.80±1.32*	17.40±1.03
Week 1	18.20±1.24	20.80±0.92	14.80±0.49*	16.80±0.73	19.20±0.58
Week 2	18.20±2.20	22.60±4.07	15.80±1.96	19.00±3.05	30.40±2.50**
Week 3	19.00±1.52	21.20±2.54	17.60±1.94	19.80±1.59	26.00±2.24*
Week 4	20.20±2.46	22.60±3.39	17.60±2.50	19.00±3.00	28.40±2.16*

Significant values are Mean ± SEM compared to control (* $P<0.05$, ** $P<0.005$,) n=5

Table 3: Mean serum alkaline phosphatase(ALP) concentration (u/l) in Rabbits treated with aqueous extract of *Cyperus esculentus* for a period of four weeks.

	GROUP A (control)	GROUP B (12.5mg/kg)	GROUP C (25mg/kg)	GROUP D (50mg/kg)	GROUP E (100mg/kg)
Baseline	19.40±0.98	24.20±1.24**	20.80±0.86	24.00±0.84**	24.00±0.32**
Week 1	20.60±0.68	21.60±1.17	23.80±1.24*	23.20±1.02	23.80±0.58*
Week 2	37.20±2.35	42.60±3.20	45.20±2.35*	51.60±1.96**	48.60±3.12*
Week 3	39.20±1.98	50.40±0.68***	48.60±0.60***	48.60±0.24***	51.00±0.45***
Week 4	49.00±1.58	48.00±2.30	46.40±0.51	46.40±0.40	50.80±0.49

Significant values are Mean ± SEM compared to control (* $P<0.05$, ** $P<0.005$, *** $P<0.001$) n=5

Table 4: Mean weights (kg) of Rabbits of the various groups treated with aqueous extract of *Cyperus esculentus* for a period of four weeks.

	Group A (control)	Group B (12.5mg/kg)	Group C (25mg/kg)	Group D (50mg/kg)	Group E (100mg/kg)
Base line	1.06±0.07	1.28±0.06	1.14±0.11	1.26±0.14	1.30±0.13
Week 1	1.18±0.04	1.18±0.12	1.12±0.07	1.22±0.12	1.28±0.14
Week 2	1.36±0.05	1.30±0.16	1.24±0.14	1.40±0.16	1.34±0.15
Week 3	1.44±0.05	1.32±0.16	1.20±0.13	1.46±0.16	1.38±0.16
Week 4	1.50±0.08	1.40±0.16	1.44±0.09	1.56±0.14	1.48±0.13

DISCUSSION

One of the initial steps in detecting damage to the liver is to determine the level of some of the enzymes in blood. Normally, the enzymes reside within the cells of the liver. However, when there is injury to the liver, these enzymes are spilled into the blood stream, raising the enzyme levels in the blood, thus signaling liver damage. The enzymes more often employed to assess hepatocellular damage are Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP). Damage to the liver tissues result in increase in the activities of these enzymes in plasma. Such increase in serum hepatic enzyme activity is known to be proportional to the extent of tissue damage (Crook, 2006).

The observed increase in ALT level can be attributed to the presence of phytochemicals which are also anti-nutrient substances. Some plant extracts have been known to possess different levels of hepatotoxicity which depends mainly on the levels of anti-nutrients inherent in the plants (Sofowora, 1993).

Phytochemical analysis of *Cyperus esculentus* revealed its content of alkaloids and saponins amongst others (Ekeanyanwu *et al.*, 2010). The mechanisms of action of alkaloids and saponins are similar and it involves complexing with cholesterol to form pores in cell membrane bilayers (Wink, 1993; Francis *et al.*, 2002). This may have been the possible mechanism by which *Cyperus esculentus* acted on the liver cells to bring about the observed increase in the level of alanine aminotransferase in this study.

The increase in serum AST concentration can also be attributed to the presence of alkaloids and saponins and the mechanism of action is similar to that described for ALT. A rise in plasma aminotransferase activity is a sensitive indicator of damage to the cytoplasm or the mitochondrial membranes of the hepatocytes. Hence, the increase in alanine and aspartate aminotransferases, which are specific for the liver cells, indicate some level of hepatotoxicity (Dial, 1995).

Increase in serum ALP is known to be a reliable indicator of cholestasis and possibly, bile duct obstruction (Wulkan, 1986). Increase in ALP could also be extrahepatic since ALP is known to be present in high concentrations in the bone, intestine, kidney, and placenta (Crook, 2006). However, an elevated ALP is also indicative that there may be active bone formation occurring, since ALP is a known by-product of osteoblast activity (Sabokbar, 1994; Crook, 2006). On the other hand, the increase in ALP serum concentration observed in this study may not have been as a result of any effect of the administered extract since a similar increase in ALP level was observed in the control group. Thus, it is suggestive that the observed increase in ALP concentration in this study may have been extra-hepatic. It is possible that this is indicative of active bone formation and growth occurring during the period. Alkaline phosphatase is a by-product of osteoblast activity (Sabokbar, 1994), therefore the rise in the ALP concentration seen in all the groups, including the control, may have been due to osteoblast activity since there was a general increase in weight and possibly bone growth.

It is noteworthy that the observable increase in weight in this study correlates well with a similar study by Ekeanyanwu *et al.* (2010). These workers attributed such increase in weight to increased feed and water intake throughout the experimental period.

From this study, it can be noted that although *Cyperus esculentus* is reported to have both nutritional and health benefits, the observed changes in the serum levels of these liver enzymes suggest that it may have some clearly definable hepatotoxic properties especially when taken in high doses and for a prolonged period. It is suggested that more studies be carried out to further elucidate the present observations.

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AUTHORS CONTRIBUTIONS

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