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Changes In Hepatopancreatic Enzyme Profile in Serum of *Costus Lucannusianus* Treated Rats

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Abstract

Medicinal plants have important contributions in the healthcare system especially in rural communities. One of such plants with a wide spectrum of patronage is *Costus lucanusianus.* This present study was carried out to investigate the subchronic effect of*Costus lucanusianus* on the activities of hepatic and pancreatic enzymes in the serum of Wistar rats administered varying doses of the plant's aqueous leaf extract. Fifteen (15) adultalbino Wistar rats weighing between 200-240g were randomly divided into three groups (n=5/group) and used. Group 1: Control (taking regular feed and water); Group 2: Experimental (administered 250mg/kg/d of *C.lucanusianus* aqueous extract daily); Group 3: Experimental (administered 500mg/kg/d of *C.lucanusianus* aqueous extractdaily). Each group was so treated for 14days and on the 15th day, the rats were sacrificed under chloroform anaesthesia after an overnight fast.Whole blood was collected and centrifuged to obtain serum which was then, used for the biochemical assay of amylase, lipase, aspartate transaminase, alanine transaminase and alkaline phosphatase using documented methods and commercial reagent kits. Results show that *Costus lucanusianus* did not produce significant changes ($p \geq 0.05$) in ALT (31.62±4.23IU/L and 32.00±2.07IU/L),AST(23.40±2.02IU/L and 23.37±2.61IU/L) and ALP(19.52±4.21IU/L and16.64±5.95IU/L) activities when compared to the control values (34.58±10.83IU/L, 33.43±3.87IU/L and 26.90±13.94IU/L respectively). Serum lipase levels did not produce any significant change($p \geq 0.05$) in group administered 250mg/kg/d (130.20±14.51IU/L) but decreased significantly($p<0.05$) in group administered 500mg/kg/d (62.60±16.54IU/L) when both compared to the control (130.60±7.09IU/L). Amylase activity levels were found toincrease significantly ($p<0.05$) in *Costus lucanusianus* administered groups (37.20±6.18IU/L and 67.40±31.15IU/L) when compared to the control group (20.40±6.07IU/L). Data indicate that *Costus lucanusianus* protects the liver but causes injury to the pancreas which affected it's exocrine activity in regulating lipase and amylase activity levels, particularly at the higher dose (5500mg/kg/d). The implication of such selective activities of *Costus lucanusianus* needs to be further understood.

Keywords: Medicinal plants, Amylase, Lipase, Aspartate transaminase, Alanine transaminase, Alkaline phosphatase, *Costus lucanusianus*

Introduction

Medicinal plants have important contributions in the healthcare system of local communities. It is the main source of medicine for the majority of the rural population (Ahmad *et al.,* 2009). The enormous popularity of medicinal plants in rural areas is due to the high cost of allopathic drugs and side effects (Marwat *et al.,* 2008).

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Herbalism could be traced to early man who probably acquired skills of healing through deliberate or accidental selection of plants and their parts (Sofowora, 1982). Herbal medicine are as effective as conventional medicines, but also has the potential to cause harmful side effects (Tapsell *et al.,* 2006). Proper double-blind clinical trials are needed to determine the safety and efficacy of each plant before they can be recommended for medical use (Vickers, 2007).

*Costus lucanusianus*J.Braun (Costaceae)is a climbing herb, found mainly in the Niger Delta region of Nigeria. It's common name is spiral ginger and it is locally called "monkey sugar cane". It is a species in the genus *Costus* which contains approximately 108 to 112 species and belongs to the family Costaceae. It is commonly used as a medicinal plant in tropical Africa (Aweke, 1994). Extracts of the plant is locally used in situations of pain, inflammation and pyrexia (Owolabi and Nworgu, 2009). Studies also show that it has antidiarrhoeal property (Owolabi *et al.,* 2007) among other traditional uses. The juice from leaves has a wide reputation in folk medicine for the treatment diarrhoea, vomiting and dysmenorrhoea (Gill, 1992). Due to worldwide usage of *Costus lucanusianus* in traditional medicine, the present study was carried out to assess the subchronic effect of varying doses of aqueous extracts of *Costus lucanusianus* on the activity of amylase and lipase (pancreatic enzymes) as well as alanine transaminase, aspartate transaminase and alkaline phosphatase (liver enzymes) in the serum of wistar rats.

Materials and Methods

Experimentalanimals: Adult Wistar rats in apparent good health with average weight of 220g and mixed sexes were used for the experiment. Fifteen Wistar rats were purchased and transported to the Laboratory Animal Centre, Faculty of Basic Medical Sciences, Delta State University, Abraka, where they were first allowed to acclimatize to environmental condition for a period of two weeks before being used for the experiment. After the acclimatization period, the experimental animals were grouped into three (3) groups of five(5) Wistar rats each to allow free and easy movement and to avoid crowdedness. The animals were grouped as follows; Group 1: Control Animals; taking regular feed and water, Group 2: Experimental Animals; taking 250mg/kg/d of *C.lucanusianus* extract and Group 3: Experimental Animals; taking 500mg/kg/d of *C.lucanusianus* extract.

Animal care and handling: The rats were kept in plastic cages under controlled condition of 12hrs light/12hrs dark cycle and allowed access to standard rat feed and water. The rats were fed on growers' mash obtained from Top Feeds Flour Mill Limited, Sapele, Delta State, Nigeria, and given clean drinking water *ad libitum*. The animals were maintained in accordance with the guidelines approved by the Animal Ethics Committee, Delta State University, Abraka, Nigeria.

Harvesting and preparation of plant extract: The mature*Costus lucanusianus* (locally called monkey sugarcane) was harvested from the rich forest of Abraka community in Ethiope East Local Government Area of Delta State, Nigeria. The leaves were identified to the species level at the Forestry Research Institute of Nigeria, Ibadan, where a voucher specimen has been deposited. The plant extract was prepared by soaking the leaves in distilled water for a period of 48hours.Thereafter, the resulting solution was oven dried to concentrate the extract before administering to the experimental animals.The *Costus lucanusianus* aqueous extract was administered as single daily dose for a period of 14 days.

Animal sacrifice and collection of specimen: Prior to the day of sacrifice,the animals were fasted for a period of 12hours. On the day of sacrifice, the animals were each anaesthesizd in a chloroform saturated chamber. When the animals became unconscious, then, the abdominal region of each rat was cut open to expose the inferior vena cava (the vein that returns blood to heart). About 5ml of blood was collected from each rat intoa plain container and centrifuged using a bucket centrifuge at 4000rpm for 10minutes in order to obtain theserum. Theserum sample obtained was stored frozen in a refrigerator until required for biochemical assay.

Amylase assay: The assay of amylase was carried out using the colorimetric method. Two hundred (200)µl of reagents were pipetted into tubes labelled "Control" and "Sample". The tubes were pre-warmed at 37ºC for 3 minutes. The spectrophotometer was then adjusted to the zero point with water at 405nm. After that, 5µl of sample was added into tube labelled "Sample" and read after 15 seconds. The reading was taken every 30 seconds for 2 minutes. The average absorbance was then determined and used to calculate the amylase activity. Assay kit was supplied by Sigma-Aldrich, St. Louis, U.S.A (CAT NO: MAK009).

Lipase assay: Test tubes were labelled, Test, Sample and Blank. Then, 1ml of chromogenic working solution and 50µl of serum were added to each test tube. Thereafter, 20µl of the esterase inhibitor solution was added to each test tube. About 100µl of substrate solution was added only to test tube Test and mixed well and immediately test tubes Test and Sample were incubated at 30ºC for 30 minutes. 2ml of reaction terminator was then added to each test tube and mixed well. About 100µl of substrate solute on was then added to test tube Sample and mixed well. The absorbances of the Test sample and the Blank sample was then measured at 412nm against Blank. Assay kit was supplied by Sigma-Aldrich,St. Louis, U.S.A (CAT NO: MAK046).

Aspartate transaminase (AST) assay: In the present method, a diazonium salt is used which selectively reacts with the oxalacetate to produce a color complex that is measured photometrically.0.5 ml of AST (SGOT) substrate was placed into test tubes labeled "Blank", "Control", and "Samples". Test tubes were warmed in 37°C heating bath for four (4) minutes. At intervals, 0.1 ml (100 µl) of samples were added into their respective tubes, gently mix, and return to 37°C heating bath for exactly ten (10) minutes. (Distilled water was used for sample blank). After ten (10) minutes, and in the same timed sequence, 0.5ml of AST (SGOT) Color Reagent was added, mix gently, and immediately returned to 37°C heating bath for another ten (10) minutes. After ten (10) minutes, 2.0 ml of 0.1 N Hydrochloric acid was added and mix by inversion. Set the wavelength of the spectrophotometer at 530 nm and the instrument was zeroed with the Blank. The absorbance of all tubes were read and recorded and used in calculating the AST activity.The biochemical assay of aspartate transaminase was carried out using commercially available kits as supplied by TECO Diagnostic, Anahein, USA (CAT NO: A561).

Alanine transaminase (ALT) assay: In this procedure ALT (SGPT) catalyzes L-alanine and α-ketoglutarate to form pyruvate and glutamate. The pyruvate is then reacted with 2, 4 dinitrophemyl-hydrazine (2,4-DNPH-ine) to form 2,4-DNPH-one. The addition of sodium hydroxide dissolves this complex, allows 2, 4-DNPH-one to be measured at 505 nm.Label test tubes "Blank", "Control", "Samples". 0.5 ml of ALT (SGPT) substrate was then transferred to each tube and placedin a 37°C heating bath for 3 - 5 minutes. At timed intervals (15 seconds),0.1 ml (100 µl) of sample was added to the correspondingly labeled tube. Mixed and immediately returned to 37°C heating bath for exactly 30 minutes. After exactly 30 minutes, 0.5 ml of ALT (SGPT) Color Reagent was added to each tube, maintaining the timed interval sequence. Mixed and returned to 37°C heating bath for exactly 10 minutes. After exactly ten (10) minutes, 2.0 ml of ALT (SGPT) Color Developer (maintaining the same timed intervals) was added. Mixed and returned to 37°C heating bath for five (5) minutes. The spectrophotometer was then zeroed with the reagent "blank" at 505nm. The absorbance of all tubes were read and recorded and used in calculating the ALT activity. The biochemical assay of alanine transaminase was carried out using commercially available kits as supplied by TECO Diagnostic, Anahein, USA (CAT NO: A526).

Alkaline phosphatase (ALP) assay: The assay of alkaline phosphatase was carried out using the method described by Kochmar and Moss(1976). In this present method, alkaline phosphatase acts upon the AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically. For each sample, 0.5ml of Alkaline phosphatase substrate was dispensed into labeled test tubes and equilibrated to 37ºC for three minutes. At intervals, 0.05ml of each standard, control and samples were added to respective tubes, mixed gently and incubated for exactly ten minutes at 37ºC (Deionized water was used for reagent blank). 2.5ml of Alkaline phosphatase color developer was then added at timed intervals and mixed well. The wavelength was then set at 590nm, and used to read and record the absorbances of the sample. Assay kit was supplied by TECO Diagnostic, Anahein, USA (CAT NO: A506).

Statistical analysis: The results were expressed as Mean±SD for n=5 mice per group. The data were evaluated by the one-way analysis of variance (ANOVA) using SPSS statistical package version 23. Difference between the means were tested with Tukey's post-hoc test for multiplecomparison and significance was considered when $p< 0.05$.

Results

The results obtained from the investigation into the subchronic effect of aqueous extract of *Costus lucanusianus* on the activities of hepatic and pancreatic enzymes in Wistar rats are presented in Table 1

Table 1: Changes in hepatopancreatic enzyme profile in serum of *Costus lucanusiannus* treated rats.

Serum Enzyme Profile (IU/L)	Treatments		
	Control	250mg/kg/d	500mg/kg/d
Liver Enzymes			
AST	33.43±3.87[a]	23.40±2.02[a]	23.37±2.61[a]
ALT	34.58±10.83[a]	31.62±4.23[a]	32.00±2.07[a]
ALP	26.90±13.94[a]	19.52±4.21[a]	16.64±5.95[a]
Pancreatic enzymes			
Amylase	20.40±6.07[a]	37.20±6.18[b]	67.40±12.15[b]
Lipase	130.60±7.09[a]	130.20±14.51[a]	62.60±16.54[b]

Values are expressed as Mean±S.D for n=5 rats per group. Values that bear another superscript on a row differ significantly ($p<0.05$).

AST = Aspartate transaminase (range: 10-40IU/L), ALT = Alanine transaminase (range:5-35IU/L),ALP = Alkaline phosphatase (range: 9-35IU/L)

Discussion

Herbal medicines are very popular in developing and underdeveloped countries.Chemical compounds in plants mediate their effect on human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. Reports that indicate that the ideal herbal drugs are very safe and free from toxic side effects are false (Calixto, 2000). Toxicological evaluations of all medicinal plants are important in order to ascertain their safety. In this study therefore, the subchronic effect of two doses (20 and 500mg/kg/d) of *Costus lucanusianus* aqueous leaf extract on serum pancreatic and hepatic enzymes were evaluated.

Amylase and lipase are digestive enzymes which are secreted by the pancreas. Amylase breaks down dietary starch while lipase aids in the digestion of dietary fats. The pancreas produces these enzymes in large quantities and secretes them into the small intestine. Diseases of the pancreas (e.g acute pancreatitis) most commonly cause elevated amylase and lipase in blood, although other disorders may lead to abnormally increased blood levels of these enzymes.During liver damage, some of it's enzymes leak into bloodstream, thereby leading to an increased activity of such enzymes in bloodstream. So, significant increase in levels of these liver marker enzymes (such as aspartate transaminase, alanine transaminase, alkaline phosphatase) would suggestdamage to liver cells. However, a decreased level of these enzymes would indicate hepatoprotection.

Table 1 shows the subchronic effect of the aqueous extract of *Costus lucanusianus* on hepatopancreatic enzyme profile in the serum of treated rats. Table 1 shows that the hepatic enzyme profile (AST, ALT and ALP) in serum of extract administered rats reduced insignificantly when compared with respective control values. However, changes in pancreatic exocrine enzymes (amylase and lipase) in serum, following extract administration indicate significant increase in serum amylase activity in a dose dependent manner. The lower dose (20mg/kg/d) maintained lipase activity value, when compared with the control, but the higher dose (500mg/kg/d) significantly ($p<0.05$) reduced lipase activity well beyond the control point (Table 1).Evidence from experimental data (Table 1) indicate that the aqueous leaf extract of *C. lucannusianus* possesses hepatoprotective property, but administration at a dose of 500mg/kg/d could induce pancreatic exocrine dysfunction.

Earlier work on the *Costus lucanusianus* extracts has shown the presence of tannins as one of the phytochemicals present in the plant along with saponins, carbohydrates and reducing sugars (Owolabi *et al.,* 2007). Plant tannins are well known natural antioxidants which could protects the liver from oxidative damage (Kumari and Jain, 2012),as also observed in this study, but such natural antioxidants could not protect the pancreas alike.

Conclusion

From the results of this study, oral administration of *Costus lucanusianus* appear to protect the hepatocytes of the experimental rats at both 250mg/kg/d and increased 500mg/kg/d doses but induces a measure of dysfuncton to the exocrine function of the pancreas. The mechanism and implication of this selective effect needs to be further understood.

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