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Cytokeratin Protein Expression, Glycogen Concentration and Liver Enzymes in the Rat's Liver After Treatment with Ethanol Stem Extract and Juice of *Costus Afer*.

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ABSTRACT

Costus afer is a medicinal plant used as a therapy for diabetes and hypertension. The study investigated the effect of crude ethanolic extract of *Costus afer* stem and its juice on the expression of CK7 cytokeratin protein, histology and some enzymes in the liver. The study is designed to assess possible toxic effects of *Costus afer* extracts consumption in the liver using rat model. The study was carried out in the Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Calabar between May and July, 2015. Twenty four (24) albino *Wistar* rats were divided into four groups of six animals each. Group one (I) served as control, Group two (II) served as experimental group and was tested with 200mg/kg body weight (low dose) of the crude ethanolic extract. Group three (III) also served as experimental group, tested with 500mg/kg body weight (high dose) of the crude ethanolic extract and Group four (IV) was tested with 500mg/kg body weight of *Costus afer* stem juice. Treatment was orally using orogastric tube for twenty eight (28) days. Result showed that there was a dose dependent reduction in the expression of cytokeratin protein, (CK7) in the test groups except those treated with the juice. Glycogen distribution was reduced with increasing dose and there was no significant effect in the serum levels of liver enzymes. In conclusion, this study suggests that the stem extract and the juice (in particular) of *Costus afer* may alter the molecular composition of the liver in a chronic consumption.

Key words: *Costus afer* extract, liver histology, CK7, glycogen

INTRODUCTION

Plant based medication has been the cheapest and commonest therapeutic weapon for the prevention and treatment of a large number of diseases. Medicinal plants are major sources of drugs¹. Natural products on the basis of their therapeutics are used in virtually all cultures². The World Health Organization (WHO) estimated that about 80% of the World's population depends on plants for their primary healthcare³. The use of herbs is seen as an alternative way to compensate for some perceived deficiencies in orthodox pharmacotherapy⁴. Herbs are common elements in ayurvedic, homeopathic, naturopathic and traditional oriented medicine⁵.

Costus afer also called "Bush cane" belongs to the *Costaceae* family (Nyananyo, 2006). It is a tropical perennial plant with creeping rhizome, commonly found in moist and shady forest of West Africa and Tropical Africa⁶. It is found in the forest belt from Senegal to Ethiopia and in the east of Tanzania, Malawi, and Angola, southern and western parts of Africa. In Nigeria, it is commonly called "Okpete" or "Okpete

Ohia" by the Ibos, in Hausa language "Kaki zuwa", in Yoruba "tete – egun" (Iwu) and "mbritem" by the Efik speaking tribe of South-south Nigeria

Costus afer as a herbal remedy is used for several ailments such as cough, sleeping sickness and stomach ache⁷. It is used as eye drops and to treat headache⁸. It is stem sap is used in the treatment of urethral discharge, venereal diseases and jaundice. Culturally, *Costus afer* is believed to have supernatural powers (oral communication). The plant is used to treat diabetes and hypertension in folklore medicine⁹. It exhibits antimicrobial property¹⁰. It has hypolipidemic activity thus having a beneficial role in the treatment of cardiovascular diseases¹¹. The stem juice and ethanol leaf extract show antinociceptive activity¹². The aqueous stem extract has significant hypoglycemic effect¹³. The methanolic extract of the stem has hepatoprotective and antioxidant property. It is a potent hepato protective agent against alcohol induced liver cirrhosis¹⁴, and paracetamol induced oxidative stress and liver damage¹⁵. The aqueous extract of the leaves altered liver enzymes in a study using *Wistar* rats¹⁶.

Costus afer juice protected the renal tissue against nitrocellular thinner induced nephrotoxicity¹⁷. Phytochemical screening of the stem of *Costus afer* revealed the presence of Alkaloids, Saponin, Steroids, Triterpenoids, Tannins, Glycosides, Carbohydrate, and Protein¹⁸. Potassium, Calcium, magnesium and phosphorus also present in the stem of *Costus afer*¹⁹. Mineral compositions of plants play significant roles in nutrition²⁰. Minerals cannot be synthesized by animals and must be provided from plants or water rich in minerals *Costus afer* contains minerals, thus its phytochemical constituents accords its great potential in herbal medicine.

Liver enzymes Alanine Aminotransferase (ALT), Aspartate aminotranferase(AST) and Alkaline Phosphatase (ALP) are used as markers to ascertain liver health particularly AST and ALT. Specific cytokeratin protein markers are useful in the diagnosis of liver disorder²¹. Considering the complexity of herbal medicines, the aim of this study was therefore to determine possible toxicity of *Costus afer extract* on the liver by assessing the expression of the cytokeratin protein, CK7, liver enzymes assay and glycogen concentration in the liver of wistar rats after consumption of *Costus afer* stem extract and juice.

Breeding of Experimental Animals

Twenty four albino wistar rats of both sexes weighing between 127 – 210 grammes (g) were used for this study. These were obtained from the Department of Zoology, University of Calabar and kept in the location of the experiment under standard conditions of room temperature (25-27°C), equal hours of dark : light cycle. The animals were allowed to acclimatize to the new environment for two weeks and were feed regularly with rat's chow from Agrofeeds Ltd, Calabar and had drinking water *ad libitum*.

Ethical Consideration

All experiments involving animals were done by approval of the committee on the Use and Care of laboratory animals in the Faculty of Basic Medical Sciences University of Calabar and all procedures were carried out in line with the guidelines of the committee and the National Academy of Science.

Preparation of Extract

The fresh stem of *Costus afer* were obtained from the University of Calabar farm.. The plant was verified by a plant taxonomist, Effa, Effa A. in the Department of Botany, University of Calabar. A sample specimen was deposited with a voucher number 559. The plant stems were chopped into pieces and air dried. They were later ground into fine powder with an electric grinder. About 7kg of the powder plant material obtained was soaked in a plastic bucket in 70% ethanol for 72 hours for thorough extraction of major components. The mixture was filtered first by using a chess cloth which produced

a filtrate followed by the filtrate being filtered again through Whattman No.1 filter paper of pore size 0.45 micrometer. The filtrate were placed in beakers and allowed to concentrate in a water bath by evaporation at 40°C to complete dryness yielding 180g of a dry mass, the extract. The juice extract was prepared by grinding a known weight of the fresh stem using an electric blender. It was then filtered to obtain the juice extract.

Experimental Protocol and Administration of Extract

The Wistar rats were divided into four groups of six rats each. The control group and the test groups. The control group was administered distilled water only. The low dose group was administered with the extract at a dose of 200mg/kg body weight, the high dose group at a dose of 500mg/kg body weight while the the fourth group was administered with the stem juice 5ml/kg body weight was administered to the rats orally through orogastric intubation, once a day for twenty eight days. This experiment lasted for twenty eight days. The animals were sacrificed at the end of the administration (After 12- 24 hours) by using ethyl ether anaesthesia. In euthanasia, an abdominal incision was made and the liver was dissected out and weighed. The liver was fixed in 10% buffered formaldehyde, prepared for paraffin sections and staining. The blocks were also sectioned and stained for the cytokeratin marker, CK7 in the liver using the Avidin Biotin complex for paraffin embedded tissues²² and glycogen distribution using periodic acid Schiff's technique.

Estimation of Liver Enzymes Activity

Blood was collected by left ventricular puncture, spun and the serum collected to assay for liver enzymes. Alanine Aminotranferase (ALT), Aspartate Aminotransferase (AST) was assayed²³ using AGAPPE diagnostic kits. Alkaline Phosphatase (ALP) was done using colorimetric method

RESULTS

Immunological Assessment: Results indicated that there was a decreased expression of the cytokeratin protein, CK7 in the test groups treated with stem extracts. This occurred with increasing dose of the stem extract compared with the control group (Figures 1-3) but the expression was observed to increase suddenly in the group treated with the juice (Figure 4).

Gycogen Distribution: The concentration of glycogen on section was observed to decrease with increasing dose of treatment compared with control. (Figs.5-8) This suggests a depletion of glycogen by the extract.

Liver function Test Result: There was an initial significant reduction in the serum levels of AST in the group treated with 200mg/kgw of stem extract but with increasing treatment the values returned to normal. ALT was not significantly affected but the

concentration of ALP was significantly elevated in the group treated with 200mg/kgw and the juice (Figure 11) when compared with control group (Figs.9-11).

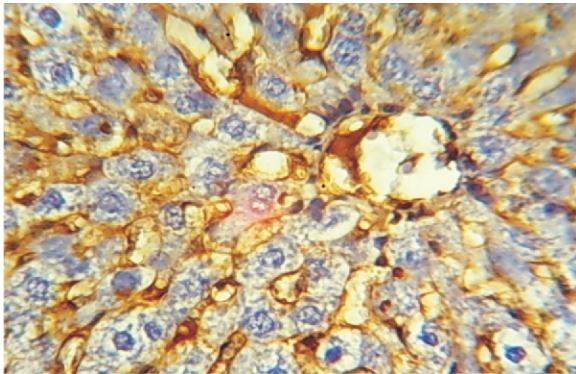


Figure 1: Section of Liver from control rat showing expression of Cytokeratin protein CK7 indicated by the Brown colouration. Immunohistochemical (IHC) staining for paraffin embedded tissues.. X400

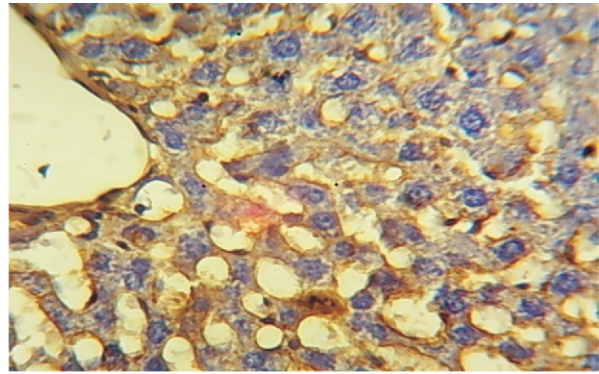


Figure 2: Section of the liver from test group treated with 200mg/kgw of *Costus afer* stem extract shows decreased expression of CK7. IHC for paraffin embedded tissues. X400

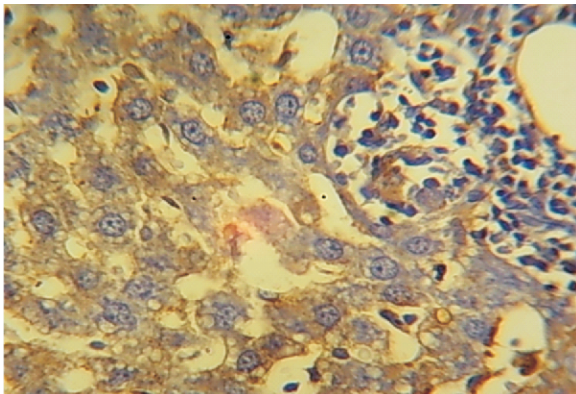


Figure 3: Section of the Liver from test group treated with 500mg/kgw of *Costus afer* stem extract shows decreased expression of CK7. X400. IHC

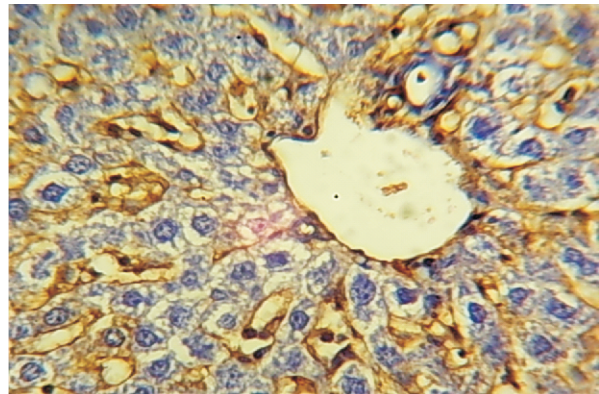


Figure 4: Section of the Liver from test group treated with 5mls/kgw of *Costus afer* Juice showing increased expression of CK7 But not different from control. X400. (IHC).

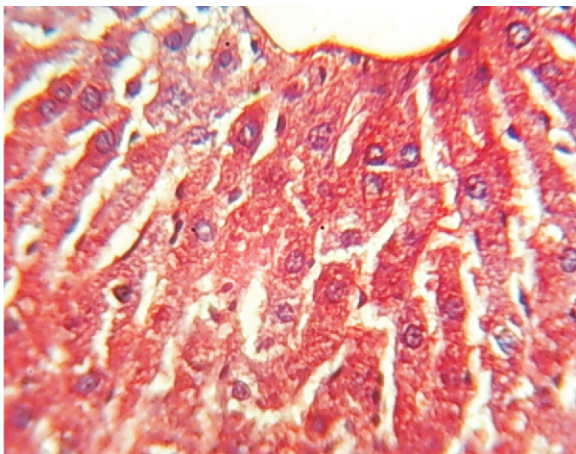


Figure 5: Section of Liver from control rat shows glycogen distribution. (magenta colour) on section. X400. Periodic Acid Schiff's (PAS) method.

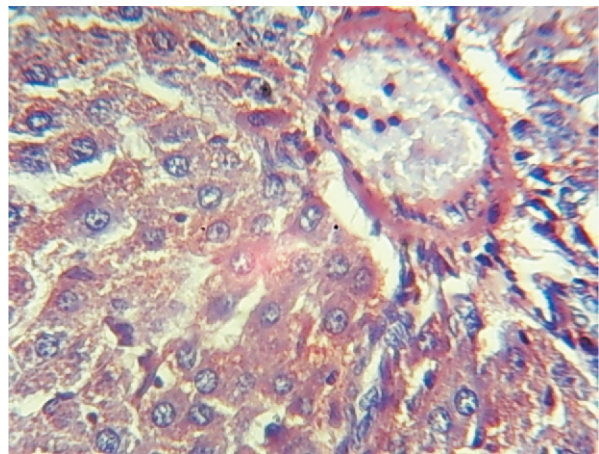


Figure 6: Section of Liver from test animals treated with 200m/kgw of *Costus afer* extract shows reduced glycogen concentration on section. X 400 PAS

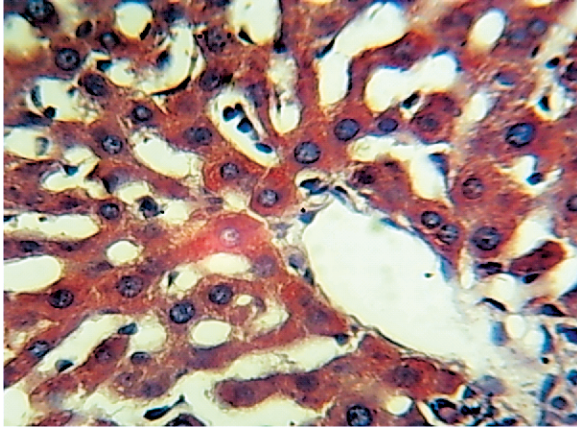


Figure 7: Section of Liver from test animals treated with 500mg/kgw of *Costus afer* extract shows decreased concentration of glycogen. X400 (PAS)

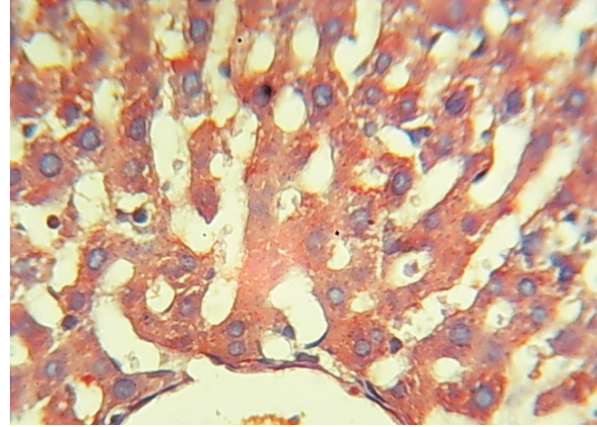


Figure 8: Section of Liver from test group treated with 5mls/kgw of *Costus afer* juice shows decreased glycogen concentration. X400 (PAS)

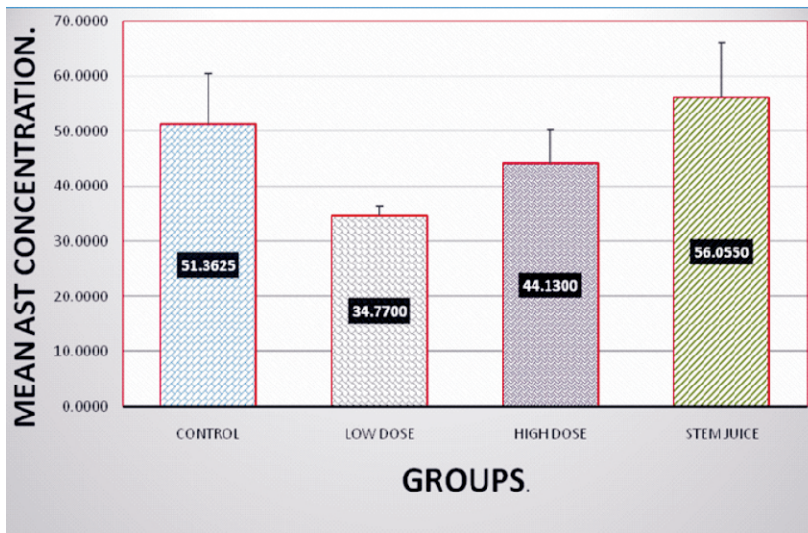


Figure 9: AST concentrations of the different experimental groups. Values are expressed as mean \pm standard error of mean. *Significantly different from control at $P < 0.05$, a= $P < 0.05$ vs low dose, b= $P < 0.05$ vs INS

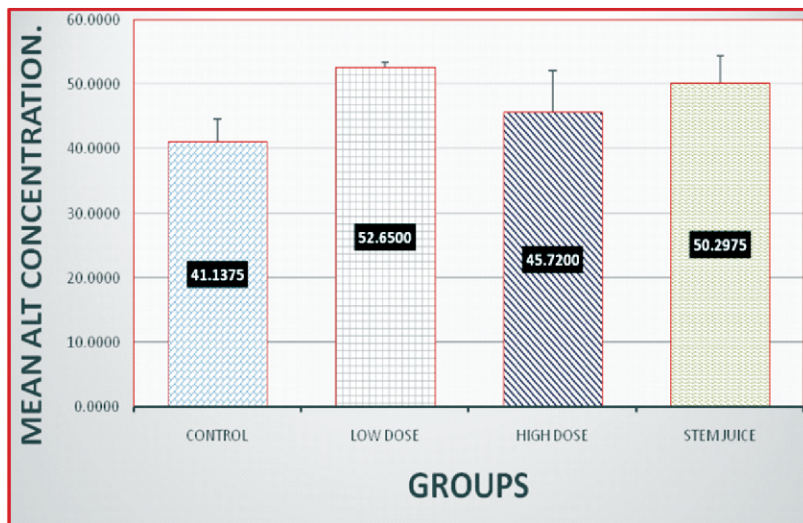


Figure 10: Chart showing ALT concentrations of the different experimental groups values are expressed as Mean \pm SEM. * Significantly different from control at $p < 0.05$. a= $P < 0.05$ vs low dose. b= $P < 0.05$ vs INS.



Figure 11: Chart showing ALP concentrations of the different experimental groups. Values are expressed as \pm standard error of mean. *Significantly different from control at $P < 0.05$. a= $P < 0.05$ vs low dose, b= $P < 0.05$ vs INS

DISCUSSION

Costus afer leaf and juice is valued in complementary alternative medicine as a herbal remedy For the management of diabetes^{24,11,13}. The result of this study suggests that *Costus afer* stem extract and juice in chronic consumption may alter the molecular composition of the cytoskeleton studied in the liver²⁵. Toxic alterations begin at the molecular levels and eventually manifests at the cellular level depending on the body's immune status. Cytokeratins are proteins of keratin-containing intermediate filaments found in the intracytoplasmic cytoskeleton of epithelial tissue (Wikipedia). The diverse and unique expression of CK7 and CK20 in carcinoma have been found to be useful in the differential diagnosis of some carcinoma of epithelial origin (Ramaekers et al., 1990). Cytokeratins, as CK7 (92%) and CK19 (83%) were found to be positive in biliary tract cancers, however, one-third of hepatocellular carcinomas also expressed CK7 (34%)²⁶. A positive expression was also observed in bile duct adenoma, mesenchymal hematoma and allograft rejection²⁷, Cirrhosis of the fibrolamella type and in perivenular hepatocytes associated with cholestatic chemistry profile and heart failure²⁸. The study of cytoskeleton protein expression has provided a valuable insight into the biliary microanatomy of the liver in health and disease. The canals of Hering are a site of origin for progenitor cells, which may repopulate the liver after cellular damage and loss²⁹ Normal bile ducts and the bile ductular reaction that occurs in many chronic liver diseases - especially chronic biliary tract disease express cytoskeleton (CK) 7 and CK19³⁰. The present study showed no increased positive expression of CK 7 cytoskeleton. This confirms previous studies that *Costus afer* stem extract is hepatoprotective^{18,9}.

The decreased distribution of glycogen may be associated with its hypolipidemic activity which has been documented¹².

Liver enzymes are supposed to be cell bound within the liver and their levels should not differ from the control. An efflux of a particular enzyme into serum is a sign of disorder. The extract had no significant effect on the levels of liver enzymes (ALT and AST) and the variances found in ALP levels may have been due to other factors outside the liver

CONCLUSION

Costus afer in chronic consumption may alter the cytoskeleton, protein expression, glycogen distribution and no significant adverse effect on liver enzymes.

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Authors' Contributions

DR AO Akpantah designed the work and did the laboratory experiments, Ita, Idongesit provided the plant material, Udo Affah, Gabriel did the literature search, Udonkang, M I. did the photomicrographic sections and Eluwa, MA did the statistical Analysis.

Conflict of Interest

The authors declare that there is no conflict of interest in this work. All authors read the work and approved of its publication. All authors confirm that part or all of this work has not been published or sent elsewhere for publication.

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