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Effect of Aqueous Extract of *Costus afer* Stems on the Liver and Cardiac Enzymes Activities of High Fat Diet Induced Hyperlipidemic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author ACC designed the study, performed the statistical analysis and managed literature searches. Author ACA wrote the protocol and wrote the first draft of the manuscript. Authors EEB and DGJ managed the analysis of the study. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The present study was carried out on the effect of aqueous extract of *Costus afer* stems on the liver and cardiac enzymes activities of diet induced hyperlipidemic rats.

Methodology: Wistar albino male rats (100-135g) were randomly distributed into 7 groups of 12 rats each. Group I was fed with standard diet as normal control rats and all the other groups were fed with high fat diet (10g eggyolk/day) for 2 weeks. The plant extract was administered orally at different concentrations of 400, 800 and 1600mg/kg b.w alone and also in combination with the reference drug, Atorvastatin® (0.26mg) to the treatment groups for four weeks. The liver and cardiac enzymes activities were observed at specific intervals (2 weeks).

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Results: Results showed a significant (p<0.05) difference in the plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities after two weeks of feeding with egg yolk supplemented diet in all the test groups when compared with the normal and hyperlipidemic control rats. After 2 and 4weeks treatment, there was a significant (p<0.05) decrease in the plasma AST, ALT and ALP activities of rats in groups IV, V and VII when compared with the normal and hyperlipidemic control rats (groups I and II). There was also a significant (p<0.05) difference in the creatine phosphokinase (CPK) activity of all the test groups when compared with the normal control rats after feeding with high fat diet for 2 weeks. After 2 and 4weeks of treatment, rats in groups III, IV and VI (HTR on atorvastatin, 0.26mg, HTR on aqueous extract, 400 mg/kg and 1600 mg) significantly decreased when compared with the normal and hyperlipidemic control rats. **Conclusions:** Hence, this shows that *Costus afer* stem extract may prevent hepatocyte injury or also maybecardioprotective against myocardial injury.

Keywords: Costus afer; egg yolk; liver enzymes; hepatocyte; atorvastatin.

1. INTRODUCTION

Medicinal plants are the most important source of life saving drugs for the majority of the world's population. Herbalism is a traditional medicinal practice based on the use of plants and plant extracts [1]. Plants form the main ingredients of medicines in traditional systems of healing and have been the source of inspiration for several major pharmaceutical drugs. These drugs have been carefully standardized for their safety and efficacy [2]. Traditional uses of any plant for medicinal purposes warrant the safety of such plant, particularly with regards to mutagenicity, nephrotoxicity, carcinogenicity and hepatotoxicity [3].

Costus afer is among 150 species of stout, perennial and rhizomatous herbs of the genus Costus [4]. Costus afer finds extensive use in folkloric medicine as a remedy for cough, rheumatic pains, sleepiness and cardiotonic [5]. Теа from the dried aerial parts is used for hypertension while the leaves are used poultry feed additives to increase as both the size and number of eggs of treated birds [6].

It is a useful medicinal plant that is highly valued for its anti-diabetic, anti-inflammatory and antiarthritic properties in South-East and South-West Nigeria [7]. The leaves are reputed to be an effective remedy for fever and malaria when boiled with leaves of *Carica papaya* (pawpaw), citrus species (orange) and bark of *Magnifera indica* (mango). The stem and juice has traditional use for treatment of cough, measles and malaria. The juice of *Costus afer* is extracted and used as an instillation for eye inflammation and defects. The young and tender leaves when chewed are believed to give strength to the weak and dehydrating patient. An infusion of the inflorescence is taken to treat stomach complaints. The powdered stems are used as an enema to treat worms and haemorrhoids.

The pulped stems taken in water are strongly diuretic. The deleafed and debarked stem is used in Nigeria against attacks of nausea and young stems are sucked by Efik to quench thirst [8]. The roots mashed to a thick paste are applied topically to abscesses and ulcers. A stem decoction (the mashed or chewed stem or the pounded fruit) mixed with sugar cane juices are taken to treat cough, respiratory problem and sore throat. The smoke of dried stem is also inhaled to treat cough [9].

The stem, seeds and rhizome of Costus afer contain several steroidal sapogenins, of which diosgenin is the most important one. The rhizome yields 0.5% diosgenin. Diosgenin is a very important raw material used as a precursor in the synthesis of a number of steroidal drugs, including corticosteroids, sex hormones, oral contraceptives and anabolic agents. The rhizomes also contain the saponins aferosides A-C, as well as dioscin and paryphyllin C and the flavonoid glycoside kaempferol 3-O-α-Lrhamnopyranoside. The last compound showed an ability to potentiate in vitro cisplatin cytotoxicity in a human colon cancer cell line [9].

Phytochemical reports indicate that the genus Costus is rich in steroidal saponins, sapogenins, oxalates, furans, furan derivatives and starches [10]. The TLC of the tubers extracted with petroleum ether and chloroform yielded lanosterol, tigogenin and diosgenin. [5] isolated costugenin and sapogenin from the chloroform extract of the plant. The present study was designed to evaluate the effect of aqueous extract of *Costus afer* on the activities of enzymes liver and cardiac in hyperlipidemic rats.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

Fresh stems of *Costus afer* were obtained from Obizi in Ezinihitte Mbaise Local Government Area of Imo State. They were authenticated by a Plant taxonomist at the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The stems were cut into pieces and sun dried. They were later ground into fine powder with the aid of a clean dry electric grinder and stored in an air tight container.

2.2 Preparation of the Stem Extract

Preparations used in traditional medicines are in cold water or hot water [11].

The plants were sun dried and ground into powder. The resultant powder was soaked in boiled water for 24hrs, after which the filtrate was filtered and the filtrate (aqueous extract) was stored for subsequent use. Ten millimetres of this extract was evaporated to dryness and the weight of the residue used to determine the concentration of the filtrate, which was in turn used to determine the dose of administration of the extract to the test animals. The extract was stored at ambient temperature

2.3 Dilutions of Drugs

The standard hypolipidemic drug Atorvastatin® (100mg) was administered at the dose of 0.26 mg/kg bodyweight dissolved in 100ml of distilled water.

2.4 Experimental Animals

Wistar albino male rats (100-135g) of body weight were obtained from Animal House of the department of Biochemistry, University of Port Harcourt, Port Harcourt Nigeria. The rats were randomly distributed into seven groups (I – VII) of 12 rats each. They were housed separately and fed ad libitum with water and growers' mash (Port Harcourt Flour Mills, Port Harcourt, Nigeria) for the 42 day period of the study and were allowed for a week to acclimatize to laboratory conditions. They were exposed to 12 hr light-dark cycle and were handled according to standard protocol. The rats were weighed, acclimatized for 7days and reweighed. The weight after 7 days

acclimatization served as the initial weight for the feeding experiment. The normal control rats was given normal feed while the six test groups received 10g raw egg yolk/40g feed for a period of 2 weeks to induce hyperlipidemia. Two weeks after inducing hyperlipidemia, the rats in group III, reference drug group received daily by oral gavages 0.26mg/kg bodyweight of Atorvastatin, group IV, V and VI rats received 400mg/kg, 800mg/kg and 1600mg/kg body weight of Costus afer extract respectively while the rats in the group VII received both 400mg/kg bodyweight of Costus afer extract and 0.26mg/kg body weight of Atorvastatin. The hyperlipidemic test control rats (Group II) and the normal control rats (Group I) received appropriate volumes of water by the same route. The dosage of administration of the extract was adapted from [12], while the 2 weeks egg yolk supplementation was a modification of 24% loading reported by [13]. Three rats were sacrificed from each group at the end of acclimatization period (Stage 1), after 2 weeks of supplementation of High fat diet (Stage 2), at 2 weeks interval for the 4 weeks of treatment with Costus aferextract (Stage 3) and at the end of the study (Stage 4). The rats were weighed biweekly. At the end of each stage the rats were weighed and fasted overnight and anaesthesized by exposure to chloroform. While under anaesthesia, they were painlessly sacrificed and blood was collected from each rat into heparin sample bottle. Blood samples were collected from overnight fasted rats using the method described by [14]. The blood samples were centrifuged at 2000 rpm for 10mins to get plasma. The plasma was collected and stored in sample containers for the blood lipid and enzyme assays.

2.5 Experimental Design

The experiment was conducted for 42 days, in which rats (n=12) are randomly divided into seven groups.

Group I: Normal control rats (NCR); fed with normal rat pellet

Group II: Hyperlipidemic control rats (HCR); fed with high fat diet (10 g eggyolk/day)

Group III: Hyperlipidemic test rats (HTR); received standard drug, Atorvastatin (0.26 mg/kg)

Group IV: Hyperlipidemic test rats (HTR); fed with high fat diet and treated with *Costus afer* extract (400 mg/kg b.wt/day)

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Group V: Hyperlipidemic test rats (HTR); fed with high fat diet and treated with *Costus afer*extract (800 mg/kg b.wt/day)

Group VI: Hyperlipidemic test rats (HTR); fed with high fat diet and treated with *Costus afer*extract (1600 mg/kg b.wt/day)

Group VII: Hyperlipidemic test rats (HTR); received aqueous extract (400 mg/kg) + standard drug, Atorvastatin (0.26 mg/kg)

2.6 Biochemical Assay

The liver and cardiac enzymes activities, alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and creatine phosphokinase assays were carried out according to the procedures described by Randox Laboratories Ltd, United Kingdom and absorbance were read using а uv-vis spectrophotometer (Model 752S, Spectrumlab).

2.7 Statistical analysis

The results were expressed as mean \pm S.D. Statistical analysis was carried out by using oneway ANOVA followed by post hoc least square difference (LSD) multiple comparison tests using SPSS 19. In all, *p*<0.05 were considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

Table 1 shows the results of the effect of aqueous extract of the stem of *Costus afer* on the plasma AST activities of normal rats and rats fed egg yolk supplemented diet, (hyperlipidemic male rats). From Table 1 it was observed there was a significant (p<0.05) difference in the plasma AST activities after two weeks of feeding with egg yolk supplemented diet in all the test groups when compared with the normal and hyperlipidmic control rats.

After 2 weeks of treatment, rats in group VII (HTR on aqueous extract, 400mg/kg and Atorvastatin (0.26 mg/kg) decreased significantly when compared with the normal and hyperlipidemic control rats. As the treatment continued to the 4th week, there was a significant (p<0.05) decrease in the plasma AST activities of rats in groups III, V and VII (HTR on atorvastatin 0.26 mg/kg, HTR on aqueous extract, 800 mg/kg and HTR on aqueous extract, 400 mg/kg and atorvastatin, 0.26 mg/kg bodyweight) when compared with the normal and hyperlipidemic control rats (groups I and II).

Table 1. Effect of aqueous extract of <i>Costus afer</i> on aspartate aminotransferase (AST) activity
(U/L) of hyperlipidemic rats

Groups		Enzyme Activity (U/L)				
		Before HFD feeding	After 14 days HFD feeding	2 weeks Treatment	4 weeks Treatment	
	Normal Control Rats (NCR)	67±1.00 ^b	67±2.00 ^b	49±9.16	43±8.18	
II	Hyperlipidemic Control Rats (HCR)	27±1.00 ^a	52±3.00 ^a	45±6.35	45±8.18	
III	Hyperlipidemic Test Rats (HTR) on Atorvastatin (0.26mg/kg)	41±2.00 ^{a,b}	59±2.00 ^{a,b}	41±0.00	24±6.11 ^{a,b}	
IV	HTR on Aqueous Extract (400mg/kg)	41±1.00 ^{a,b}	36±2.00 ^{a,b}	41±9.23	45±3.46	
V	HTR on Aqueous Extract (800mg/kg)	27±1.00 ^a	37±3.00 ^a	40±7.23	30±1.00 ^b	
VI	HTR on Aqueous Extract (1600mg/kg)	30±2.00 ^a	47±1.00 ^{a,b}	39±10.96	34±11.54	
VII	HTR on Aqueous Extract (400mg/kg) + Atorvastatin (0.26mg/kg)	41±3.00 ^{a,b}	31±2.00 ^{a,b}	28±6.65 ^{a,b}	25±7.76 ^{a,b}	

Values are expressed as mean ± SD (n=3), per group/week. Values with superscript letter (a) are significantly different at p<0.05 when compared to group I (normal control rats). Values with superscript letter (b) are significantly different at p<0.05 when compared to group II (hyperlipidemic control rats). Values without superscripts indicate no significant difference when compared with the normal and hyperlipidemic control groups (groups I and II)

Table 2 shows the results of the effect of aqueous extract of the stem of *Costus afer* on the plasma ALT activities of normal rats and rats fed egg yolk supplemented diet, (hyperlipidemic male rats).

From Table 2, it was observed that there was a significant (p<0.05) increase in the plasma ALT activities of rats in groups IV, V and VI after feeding with egg yolk supplemented diet for 2 weeks when compared to the normal and hyperlipdemic control rats.

After 2 weeks treatment, the plasma ALT activities of rats in groups III, IV and VII with the 35±2.88. 35±2.51 values and 29±11.71 decreased (p<0.05) significantly when compared with the normal and hyperlipidemic control rats the values 51±2.30 and 53±17.05 with respectively. After 4 weeks treatment only rats in group VII decreased p<0.05 significantly when compared with the normal and hyperlipidemic control rats.

Table 3 shows the results of the effect of aqueous extract of the stem of *Costus afer* on the plasma ALP activities of normal rats and rats fed egg yolk supplemented diet, (hyperlipidemic male rats). It was observed that after 2 weeks of feeding with egg yolk supplemented diet, the plasma ALP activities of all the test groups (rats in groups III, IV, V, VI and VII) were significantly different from the rats in normal and hyperlipidemic control groups.

After 2 weeks of treatment, ALP activity of rats in groups IV, V and VI significantly (p<0.05) increased with the values 34 ± 9.50 , 38 ± 1.00 and 37 ± 4.61 when compared to hyperlipidemic control rats with the value 23 ± 2.51 . After 4 weeks treatment, only rats in group V (HTR on aqueous extract, 800mg/kg) had a significant (p<0.05) decrease in their plasma ALP activities when compared with the normal and hyperlipidemic control rats.

Table 4 shows the results of the effect of aqueous extract of the stem of Costus afer on the creatine phosphokinase activity of normal rats and rats fed egg yolk supplemented diet, (hyperlipidemic male rats). There was a significant (p<0.05) difference in the CPK activity of all the test groups when compared with the normal control rats after feeding with high fat diet for 2 weeks. After 2 and 4weeks of treatment, rats in groups III, IV and VI (HTR on atorvastatin, 0.26mg, HTR on aqueous extract, 400mg/kg and 1600mg) significantly decreased when compared with the normal and hyperlipidemic control rats.

Table 2. Effect of aqueous extract of <i>Costus afer</i> on alanine aminotransferase (ALT) activity
(U/L) of hyperlipidemic rats

Groups		Enzyme Activity (U/L)			
		Before HFD feeding	After 14 days HFD feeding	2 weeks Treatment	4 weeks Treatment
Ι	Normal Control Rats (NCR)	21±1.00 ^b	12±1.00	51±2.30	16±7.50
П	Hyperlipidemic Control Rats (HCR)	12±1.00 ^a	12±2.00	53±17.05	17±0.00
III	Hyperlipidemic Test Rats (HTR) on Atorvastatin (0.26 mg/kg)	25±2.00 ^{a,b}	12±1.00	35±2.88 ^{a,b}	17±0.00
IV	HTR on Aqueous Extract (400 mg/kg)	17±3.00 ^{a,b}	17±1.00 ^{a,b}	35±2.51 ^{a,b}	14±5.19
V	HTR on Aqueous Extract (800 mg/kg)	17±2.00 ^{a,b}	17±3.00 ^{a,b}	42±2.88	12±1.00
VI	HTR on Aqueous Extract (1600 mg/kg)	30±1.00 ^{a,b}	52±1.00 ^{a,b}	46±2.88	11±6.55
VII	HTR on Aqueous Extract (400 mg/kg) + Atorvastatin (0.26 mg/kg)	17±1.00 ^{a,b}	12±3.00	29±11.71 ^{a,b}	4±0.00 ^{a,b}

Values are expressed as mean ± SD (n=3), per group/week. Values with superscript letter (a) are significantly different at p<0.05 when compared to group I (normal control rats). Values with superscript letter (b) are significantly different at p<0.05 when compared to group II (hyperlipidemic control rats). Values without superscripts indicate no significant difference when compared with the normal and hyperlipidemic control groups I (groups I and II)

Groups		Enzyme Activity (U/L)				
		Before HFD	O After 14	2 weeks	4 weeks	
		feeding	days HFD feeding	Treatment	Treatment	
Ι	Normal Control Rats (NCR)	59±3.00 ^b	38±1.00 [⊳]	32±5.13 [⊳]	51±1.00	
Ш	Hyperlipidemic Control Rats (HCR)	55±2.00 ^a	65±2.00 ^a	23±2.51 ^a	46±6.65	
III	Hyperlipidemic Test Rats (HTR) on Atorvastatin (0.26 mg/kg)	16±1.00 ^{a,b}	44±2.50 ^{a,b}	29±5.50	48±2.88	
IV	HTR on Aqueous Extract (400 mg/kg)	49±2.00 ^{a b}	44±1.00 ^{a,b}	34±9.50 ^b	49±7.00	
V	HTR on Aqueous Extract (800 mg/kg)	39±2.00 ^{a,b}	52±1.00 ^{a,b}	38±1.00 ^b	30±3.00 ^{a,b}	
VI	HTR on Aqueous Extract (1600 mg/kg)	40±1.00 ^{a,b}	42±1.00 ^{a,b}	37±4.61 ^b	50±2.08	
	HTR on Aqueous Extract (400 mg/kg) + Atorvastatin (0.26 mg/kg)		43±1.00 ^{a,b}	31±3.6	50±4.35	

Table 3. Effect of aqueous extract of *Costus afer* on alkaline phosphatase (ALP) activity (U/L) of hyperlipidemic rats

Values are expressed as mean ± SD (n=3), per group/week. Values with superscript letter (a) are significantly different at p<0.05 when compared to group I (normal control rats). Values with superscript letter (b) are significantly different at p<0.05 when compared to group II (hyperlipidemic control rats). Values without superscripts indicate no significant difference when compared with the normal and hyperlipidemic control groups (groups I and II)

Table 4. Effect of aqueous extract of Costus afer on Creatine phosphokinase (CPK) activity (U/L) of hyperlipidemic rats

Groups		Enzyme Activity (U/L)			
		Before HFD feeding	After 14 days HFD feeding	2 weeks Treatment	4 weeks Treatment
Ι	Normal Control Rats (NCR)	2.5±0.10	2.0±0.20 ^b	3.3±0.69	2.3±0.34
11	Hyperlipidemic Control Rats (HCR)	2.5±0.20	2.5±0.10 ^a	2.5±0.00	2.3±0.34
	Hyperlipidemic Test Rats (HTR) on Atorvastatin (0.26 mg/kg)	2.5±0.30	2.5±0.15 ^ª	3.7±0.00 ^b	2.3±0.34
V	HTR on Aqueous Extract (400 mg/kg)	2.5±0.10	2.5±0.25 ^ª	3.2±0.61	3.3±0.40 ^{a,b}
V	HTR on Aqueous Extract (800 mg/kg)	2.5±0.30	2.6±0.10 ^a	2.9±0.83	2.6±0.36
VI	HTR on Aqueous Extract (1600 mg/kg)	2.5±0.00	2.7±0.10 ^a	3.7±0.00 ^b	4.1±0.28 ^{a,b}
VII	HTR on Aqueous Extract (400 mg/kg) + Atorvastatin (0.2 6 mg/kg)	2.4±0.10	2.5±0.05 ^ª	2.9±0.69	2.9±0.34

Values are expressed as mean ± SD (n=3), per group/week. Values with superscript letter (a) are significantly different at p<0.05 when compared to group I (normal control rats). Values with superscript letter (b) are significantly different at p<0.05 when compared to group II (hyperlipidemic control rats). Values without superscripts indicate no significant difference when compared with the normal and hyperlipidemic control groups (groups I and II)

3.2 Discussion

Liver enzymes are well known biomarkers for the prediction of liver toxicity and as such, have been used in scientific report [15,16]. Available evidence show that damage to liver cells results in elevations of these enzymes in the serum and the measurement of enzyme activities is of clinical and toxicological significance in determining liver damage by toxicants or in diseased conditions [17,18]. The level of these enzymes in the blood is directly related to the extent of the tissue damage.

3.2.1 Effect of aqueous extract of *Costus afer*on aspartate aminotransferase (AST) activity (U/L) of hyperlipidemic rats

The result of aspartate aminotransferase (AST) activity in the rats after 2 weeks of high fat diet showed that rats in groups (II, III, IV, V & VI) were significantly (p<0.05) different from rats in group I(normal control rats). However there was a decrease after 2 weeks & 4 weeks of treating with the plant extract in all the test groups. The values of AST for the rats in all the test groups were not significantly different from each other. In

other words administration of aqueous extract was dose dependent after 2 weeks of treatment and it compared favourably with the activity of the standard drug (Atorvastatin) which was progressive to the 4th week of treatment. Therefore this indicates that the extract did not have an effect on the enzyme activity and thus it may not cause hepatic injury.

3.2.2 Effect of aqueous extract of *Costus afer* on alanine aminotransferase (ALT) activity (U/L) of hyperlipidemic rats

The levels of ALT were used to monitor possible adverse effect of high fat diets on liver and biliary tract functions [19]. ALT is a reliable indicator of liver necrosis in small animals [20]. ALT another enzyme used as a marker of hepatic function after 2 weeks of feeding with high fat diet from the values obtained for rats in groups IV, V, VI were significantly (p<0.05) higher than the normal and hyperlipidemic control rats, Table 2. After treatment with the plant extract for 2 and 4 weeks there was a reduction in all the test groups when compared to their test controls. Hence, the reduction of ALT activities in the extract treated group suggested that the extracts may protect hepatocyte injury.

3.2.3 Effect of aqueous extract of *Costus afer* on alkaline phosphatase (ALP) activity (U/L) of hyperlipidemic rats

Alkaline phosphatase, a hydrolase enzyme responsible for removing phosphate group from many types of molecules including nucleotides, proteins etc is another marker of hepatic function [21]. The result thus further shows a significant (p<0.05) difference in the plasma ALP activities of rats in all the test groups when compared to the normal and hyperlipidemic control rats. After 2 weeks of treatment groups IV, V and VI (HTR on aqueous extract, 400, 800 and 1600 mg/kg b.w respectively) were significantly (p<0.05) higher than the hyperlipidemic control rats (group II). Rats in group III increased but was not significantly different (p<0.05) from normal and hyperlipidemic control rats. Table 3 shows that after 4 weeks of treatment with the plant extract. the ALP activities of rats in group V was significantly (p<0.05) lower than those in normal and hyperlipidemic control rats (group I). And there was no significant difference from other test groups when compared to the normal and hyperlipidemic control rats (group I & II), indicating that the Costus afer does not have an effect on the activity, thus it may not cause hepatic injury.

3.2.4 Effect of aqueous extract of *Costus afer* on Creatine phosphokinase (CPK) activity (U/L) of hyperlipidemic rats

Creatine phosphokinase is an enzyme used for diagnosing myocardial infarction in humans. It is an enzyme found mainly in the heart, brain and skeletal muscle. When there is a muscle damage CPK enzyme leaks into the bloodstream. In the cells the cytosolic CPK enzymes consist of two subunits which can be either B (brain type) or M (muscle type). Elevation of CPK is an indication of damage to muscle. It is therefore indicative of injury, myocardial Infarction & myocarditis. CPK activity is a more sensitive indication in early stage of myocardial ischemia [22]. In this study it shows that after 2 weeks of feeding with high fat diet the CPK activity of all the test groups was significantly(p<0.05) higher than the normal control rats but non significantly different from the hyperlipidemic control rats. There was a significant increase in the CPK activity of rats in groups III & VI after 2 weeks of treatment compared to the hyperlipidemic control.

4. CONCLUSION

The result of the present study shows that aqueous stem extract of *Costus afer* at the doses investigated poses no significant hepatotoxic or cardiotoxic effect. Hence, this shows that *Costus afer* stem extract may be suggested to possess hepatoprotective or cardioprotective potency.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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