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Effect of Aqueous Extract of *Costus afer* Stems on the Serum Proteins and Bilirubin Levels 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. Authors ACA and ORI wrote the protocol, wrote the first draft of the manuscript and manage the analysis of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The effect of aqueous extract of *Costus afer* stems on total protein, albumin,globulin, total and conjugated bilirubin levels in diet induced hyperlipidemic rats were studied.
Methodology: Wistar albino male rats (100-135 g) 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 (10 g 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 serum proteins and bilirubin were observed at specific intervals (2 weeks).
Results: The results revealed significant ($p<0.05$) increase in the total protein, albumin and globulin concentration after 2 weeks of feeding with high fat diet in rats which were in groups IV and V as

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compared to normal control rats. Treatment of rats with plant extract showed no significant difference in the total protein concentration of hyperlipidemic test rats while the albumin concentration of rats in group VII increased significantly when compared to the normal and hyperlipidemic control rats. There was no significant difference in the globulin concentration of hyperlipidemic treated rats. After 2nd and 4th week of treatment, the total bilirubin concentrations of rats in groups V and VI (HTR on aqueous extract, 1600 mg/kg and HTR on aqueous extract, 800 mg/kg) decreased significantly when compared with normal and hyperlipidemic control rats. After 2 weeks of treatment, the conjugate bilirubin concentration of rats in group VI (HTR on aqueous extract, 1600 mg/kg) significantly ($p<0.05$) decreased when compared to the hyperlipidemic control rats.

Conclusions: Hence, this shows that *Costus afer* stem extract does not have any deleterious effect on tissues and on the analyzed parameters.

Keywords: Costus afer; egg yolk; total protein; albumin; bilirubin; atorvastatin.

1. INTRODUCTION

Medicinal plants are plants which one or more of their organ contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [1,2]. A number of plants have being used in traditional medicine for many years. Plants have developed the ability to synthesize chemical substances that help them defend themselves against attack from a wide variety of predators such as insects, fungi and herbivorous mammals [3].

It is generally known that the consumption of a variety of local herbs and vegetables by man contributes significantly to the improvement of human health, in terms of prevention, and or cure of diseases because plants have long served as a useful and natural source of the therapeutic agents [4].

Costus afer is among 150 species of stout, perennial and rhizomatous herbs of the genus *Costus* [5]. *Costus afer* finds extensive use in folkloric medicine as a remedy for cough, rheumatic pains, sleepiness and cardiotonic [6]. Tea from the dried aerial parts is used for hypertension while the leaves are used as poultry feed additives to increase both the size and number of eggs of treated birds [7].

It is a useful medicinal plant that is highly valued for its anti-diabetic, anti-inflammatory and anti-arthritic properties in South-East and South-West Nigeria [8]. 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 [9]. 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 [10].

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 saponinsaferosides A–C, as well as dioscin and paryphyllin C and the flavonoid glycoside kaempferol 3-O-α-L-rhamnopyranoside. The last compound showed an ability to potentiate in vitro cisplatin cytotoxicity in a human colon cancer cell line [10].

Phytochemical reports indicate that the genus Costus is rich in steroidal saponins, sapogenins, oxalates, furans, furan derivatives and starches [11]. The TLC of the tubers extracted with petroleum ether and chloroform yielded

lanosterol, tigogenin and diosgenin. [6] 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 on total protein, albumin, globulin, total and conjugate bilirubin 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

The plants were sun dried and ground into powder. The resultant powder was soaked in boiled water for 24 hrs, 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.

2.3 Dilutions of Drugs

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

2.4 Experimental Animals

Wistar albino male rats (100-135 g) 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 7 days and reweighed. The weight after 7days acclimatization served as the initial weight for the feeding experiment. The normal control rats was given normal feed while the six test groups received 10 g raw egg yolk/40 g 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 0.26 mg/kg bodyweight of Atorvastatin, group IV, V and VI rats received 400 mg/kg, 800mg/kg and 1600 mg/kg body weight of *Costus afer* extract respectively while the rats in the group VII received both 400 mg/kg bodyweight of *Costus afer* extract and 0.26 mg/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 afer* extract (Stage 3) and at the end of the study (Stage 4). The rats were weighed bi-weekly. 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 jugular vein 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 10 mins 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)

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

Determination of total protein, albumin, and globulin were done according to the method described by [15]. The total and conjugate bilirubin assays were carried out according to the procedures described by Randox Laboratories Ltd, United Kingdom.

2.7 Statistical Analysis

The results were expressed as mean ± S.D. Statistical analysis was carried out by using one-way 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 total protein concentrations of normal rats and rats fed egg yolk supplemented diet, (hyperlipidemic rats).

From Table 1, it was observed that after feeding with high fat diet for 2 weeks there was a significant ($p<0.05$) decrease in the total protein concentration of rats in groups V, VI and VII when compared with the normal and hyperlipidemic control rats. There was no significant ($p<0.05$) difference in the total protein concentration among the groups treated for 2 and 4 weeks respectively.

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 albumin concentrations of normal rats and rats fed egg yolk supplemented diet, (hyperlipidemic male rats).

After 2 weeks of feeding with high fat diet, the albumin concentrations of rats in group V and VI increased ($p<0.05$) significantly when compared with the normal and hyperlipidemic control rats. Only rats in group VII (HTR on aqueous extract 400mg/kg and atorvastatin, 0.26mg) decreased ($p<0.05$) significantly when compared with the normal and hyperlipidemic control rats after 2 weeks treatment. There was no significant difference in the albumin concentration after 4 weeks of treatment when compared with the normal and hyperlipidemic control rats.

Table 1. Effect of aqueous extract of *Costus afer* on Total protein (g/L) concentrations of hyperlipidemic rats

Groups		Total protein (g/L)			
		Before HFD feeding	**After 14 days HFD feeding**	**2 Weeks treatment**	**4 Weeks treatment**
I	Normal Control Rats (NCR)	96±2.00[b]	91±1.00	72±6.35	78±11.93
II	Hyperlipidemic Control Rats (HCR)	84±4.00[a]	95±2.00	76±0.00	85±9.53
III	Hyperlipidemic Test Rats (HTR) on Atorvastatin (0.26 mg/kg)	79±3.00[a]	91±3.00	81±5.00	79±5.50
IV	HTR on Aqueous Extract(400 mg/kg)	82±3.00[a]	89±5.00	78±0.57	85±8.50
V	HTR on Aqueous Extract (800 mg/kg)	80±5.00[a]	79±0.57[a,b]	80±1.00	82±1.00
VI	HTR on Aqueous Extract (1600 mg/kg)	82±2.00[a]	72±3.00[a,b]	82±15.14	83±11.15
VII	HTR on Aqueous Extract (400 mg/kg) + Atorvastatin (0.26 mg/kg)	84±1.00[a]	84±4.00[a,b]	82±0.00	77±10.69

Table 2. Effect of aqueous extract of *Costus afer* on Albumin (g/L) concentrations of hyperlipidemic rats

Groups		Albumin (g/L)			
		Before HFD feeding	After 14 days HFD feeding	2 Weeks treatment	4 Weeks treatment
I	Normal Control Rats (NCR)	29±2.00	32±3.00	49±3.05	39±2.08
II	Hyperlipidemic Control Rats (HCR)	30±3.00	32±1.00	50±1.15	37±1.00
III	Hyperlipidemic Test Rats (HTR) on Atorvastatin (0.26 mg/kg)	47±2.00[a,b]	32±2.00	49±2.08	40±0.00
IV	HTR on Aqueous Extract (400 mg/kg)	36±2.00[a,b]	29±1.00	48±3.51	37±2.51
V	HTR on Aqueous Extract (800 mg/kg)	31±1.52	36±2.00[a,b]	47±2.00	37±1.52
VI	HTR on Aqueous Extract (1600 mg/kg)	35±3.00[a,b]	45±3.00[a,b]	46±1.15	36±1.00
VII	HTR on Aqueous Extract (400 mg/kg) + Atorvastatin (0.26 mg/kg)	38±0.57[a,b]	29±2.00	45±1.00[a,b]	36±2.88

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 3 shows the results of the effect of aqueous extract of the stem of *Costus afer* on the globulin concentrations of normal rats and rats fed egg yolk supplemented diet, (hyperlipidemic male rats). There was a significant (p<0.05) decrease in the globulin concentration of rats in groups V, VI and VII when compared with the rats in the normal and hyperlipidemic control groups after 2 weeks of feeding with high fat diet. The globulin concentrations of rats in groups VI and VII (HTR on aqueous extract, 1600mg/kg and HTR on aqueous extract, 400mg/kg and atorvastatin, 0.26mg/kg) increased (p<0.05) when compared with the normal control rats (group I). There was no significant (p<0.05) difference in the globulin concentration of all the test groups when compared with the normal and hyperlipidemic control rats (groups I and II).

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 3. Effect of aqueous extract of *Costus afer* on globulin (g/L) concentrations of hyperlipidemic rats

Groups		Globulin (g/L)			
		Before HFD feeding	After 14 days HFD feeding	2 Weeks treatment	4 Weeks treatment
I	Normal Control Rats (NCR)	67±0.00[b]	59±2.00	22±6.50	39±13.74
II	Hyperlipidemic Control Rats (HCR)	54±1.00[a]	63±1.00	26±1.15	48±9.53
III	Hyperlipidemic Test Rats (HTR) on Atorvastatin (0.26 mg/kg)	32±2.64[a,b]	59±5.00	32±5.85	39±5.50
IV	HTR on Aqueous Extract (400 mg/kg)	46±1.00[a,b]	60±6.00	31±4.04	48±9.84
V	HTR on Aqueous Extract (800 mg/kg)	49±6.50[a,b]	43±2.51[a,b]	33±1.00	45±0.57
VI	HTR on Aqueous Extract (1600 mg/kg)	47±2.64[a,b]	27±6.00[a,b]	37±14.46[a]	47±10.40
VII	HTR on Aqueous Extract (400 mg/kg) + Atorvastatin (0.26 mg/kg)	47±0.57[a,b]	55±6.00[b]	37±1.00 [a]	40±9.71

Table 4 shows the results of the effect of aqueous extract of the stem of *Costus afer* on the total bilirubin concentrations of normal rats and rats fed egg yolk supplemented diet, (hyperlipidemic male rats).There was a significant ($p<0.05$) difference in the total bilirubin concentration of all the test groups (rats in groups III, IV, V, VI and VII) after feeding on high fat diet for 2 weeks when compared with the normal and hyperlipidemic control rats.

After 2nd and 4th week of treatment, the total bilirubin concentrations of rats in groups V and VI (HTR on aqueous extract, 1600 mg/kg and HTR on aqueous extract, 800 mg/kg) decreased significantly when compared with normal and hyperlipidemic control 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 5 shows the results of the effect of aqueous extract of the stem of *Costus afer* on the conjugate bilirubin concentrations of normal rats and rats fed egg yolk supplemented diet, (hyperlipidemic male rats). There was a significant ($p<0.05$) increase in the conjugate bilirubin concentration of rats in groups IV, V, VI and VII when compared with the normal and hyperlipidemic control rats. After 2 weeks of treatment, the conjugate bilirubin concentration of rats in group VI (HTR on aqueous extract, 1600mg/kg) significantly ($p<0.05$) decreased when compared to the hyperlipidemic control rats.

Table 4. Effect of aqueous extract of *Costus afer* on Total bilirubin (µmol/l) concentrations of hyperlipidemic rats

Groups		Total Bilirubin (µmol/L)			
		Before HFD feeding	**After 14 days HFD feeding**	**2 Weeks treatment**	**4 Weeks treatment**
I	Normal Control Rats (NCR)	24.0±1.10[b]	11.1±0.35[b]	8.0±2.13	12.1±4.30
II	Hyperlipidemic Control Rats (HCR)	12.9±0.20[a]	9.3±0.10[a]	8.0±5.31	10.8±5.20
III	Hyperlipidemic Test Rats (HTR) on Atorvastatin (0.26 mg/kg)	3.8±0.10[a,b]	11.1±0.25[b]	6.2±2.80	9.9±2.84
IV	HTR on Aqueous Extract (400 mg/kg)	14.8±0.10[a,b]	12.9±0.10[a,b]	9.3±1.85	11.7±3.85
V	HTR on Aqueous Extract (800 mg/kg)	9.3±0.30[a,b]	13.0±1.00[a,b]	9.0±0.30	8.0±0.20[a,b]
VI	HTR on Aqueous Extract (1600 mg/kg)	5.0±1.00[a,b]	11.1±0.10[b]	1.9±0.00[a,b]	15.0±1.73
VII	HTR on Aqueous Extract (400 mg/kg) + Atorvastatin (0.26 mg/kg)	14.8±0.35[a,b]	14.8±0.20[a,b]	5.6±4.80	12.9±1.90

Table 5. Effect of aqueous extract of *Costus afer* on Conjugate bilirubin (µmol/l) concentrations of hyperlipidemic rats

Groups		Conjugate bilirubin (µmol/L)			
		Before HFD feeding	**After 14 days HFD feeding**	**2 Weeks treatment**	**4 Weeks treatment**
I	Normal Control Rats (NCR)	10.0±2.0[b]	2.0±0.20	4.0±1.38	4.9±2.45
II	Hyperlipidemic Control Rats (HCR)	2.5±0.05[a]	1.8±0.35	5.4±3.40	5.7±3.70[a]
III	Hyperlipidemic Test Rats (HTR) on Atorvastatin (0.26 mg/kg)	2.4±0.20[a]	2.0±0.30	3.2±1.38	4.9±2.45
IV	HTR on Aqueous Extract (400 mg/kg)	9.7±0.20[b]	2.5±0.10[b]	4.9±2.50	6.5±3.72
V	HTR on Aqueous Extract (800 mg/kg)	4.9±0.30[a,b]	7.3±0.30[a,b]	3.5±0.10	4.0±0.25
VI	HTR on Aqueous Extract (1600 mg/kg)	4.2±0.10[a,b]	7.4±0.30[a,b]	1.5±0.00[b]	8.1±2.77
VII	HTR on Aqueous Extract (400 mg/kg) + Atorvastatin (0.26 mg/kg)	9.7±0.30[b]	3.5±0.40[a,b]	3.8±3.17	8.9±1.38

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

Proteins play a major role in maintaining the delicate acid-alkaline balance of your blood. Total protein, albumin and globulin levels assay are known to be useful in assessing the functional integrity of the liver. The total protein represents the sum of albumin and globulins.

Total protein may be decreased due to malnutrition and malabsorption, liver disease, diarrhoea (loss of protein through the GI tract), severe burns (loss of protein through the skin), hormone imbalances that favour breakdown of tissue, loss through the urine in severe kidney disease (proteinuria), low albumin and globulins [16]. Albumin, total bilirubin and globulin are mixtures of molecules that can be used to evaluate the normal functioning of the liver of animals [17].

Bilirubin is an important metabolic product of blood with biological and diagnostic values. Bilirubin is a metabolic end product of lysis of erythrocytes. The reticulo-endothelial system catabolizes haemoglobin into free iron, globin and bilverdin which is rapidly converted to bilirubin.

3.2.1 Effect of aqueous extract of *Costus afer* on total protein, albumin and globulin concentration of hyperlipidemic rats

Table 1, 2 & 3 shows the effect of *Costus afer* on total protein, albumin and globulin concentration of hyperlipidemic rats. The results revealed significant ($p<0.05$) increase in the total protein, albumin and globulin concentration after 2 weeks of feeding with high fat diet in rats which were in groups IV and V as compared to normal control rats. This shows that for albumin, increased serum cholesterol raised LDL and VLDL levels which could alter the permeability of the glomerular basement membrane by neutralizing its negative charges [18] resulting in albumin molecules being excreted. In this study, treatment of rats with plant extract showed no significant difference in the total protein concentration of hyperlipidemic test rats while the albumin concentration of rats in group VII increased significantly when compared to the normal and hyperlipidemic control rats. There was no significant difference in the globulin concentration of hyperlipidemic treated rats. This implies that the plant does not have any deleterious effect on tissues.

3.2.2 Effect of aqueous extract of *Costus afer* on total and conjugate bilirubin concentration of hyperlipidemic rats

Bilirubin is a metabolic end product of lysis of erythrocytes. The reticulo-endothelial system catabolizes haemoglobin into free iron, globin and bilverdin which is rapidly converted to bilirubin. Unconjugated bilirubin is poorly soluble in the serum therefore it is transported to liver bound to albumin. In the liver, Glucoronyl transferase conjugates bilirubin with two molecules of glucuronic acid, forming bilirubin diglucuronide. This form of bilirubin is highly soluble in serum and is known as direct or hepatic bilirubin while unconjugated form is known as indirect or prehepatic bilirubin. In this study Table 4 and Table 5 shows that after 2 weeks of feeding with high fat diet, the bilirubin level of rats in all the test groups significantly ($p<0.05$) increased when compared to the normal and hyperlipidemic control rats. After 2 weeks treatment rats in Group IV significantly ($p<0.05$) decreased when compared to the normal and hyperlipidemic control rats in Table 4 while in Table 5 rats in group VI (HTR on aqueous extract, 1600mg/kg b.w) significantly decreased when compared to the hyperlipidemic control rats. After 4 weeks of treatment the plant extract was able to decrease the total bilirubin level.

4. CONCLUSION

This study has revealed that that the aqueous stem extract of *Costus afer* at the doses investigated may be suggested to possess hepatoprotective potency because there were no significant hepatotoxic effects in the hyperlipidemic rats.

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.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ. Health Perspect. 2001;109: 69-75.
2. WHO. Principles of traditional medicine. 8th Edn., New York: American Press. 2003;124-126.
3. Lai PK, Roy J. Antimicrobial and chemopreventive properties of herbs and spices. Curr. Med. Chem. 2004;11:1451-1460.
4. Chevellier A. The encylopedia of medical plant. London: Dorling Kindersley Ltd; (online); 1996. Available:http://www.chclibrary.org/plant.html. [Google Scholar]
5. Edeoga HO, Okoli BE. Chromosome numbers of *Costus lucanusianus* (Costaceae) in Nigeria. Folia Geobotanica. 2000;35:315-318.
6. Iwu MM. Steroidal constituents of *Costus afer*. Planta Medica. 1982;44:413-415.
7. Ekemini BI, Uwemedimo EO. Phytochemical profile, adsorptive and inhibitive behaviour of *Costus afer* extracts on aluminium corrosion in hydrochloric acid. Der Chemica Sinica. 2012;3(6):1394-1405.
8. Soladoye MO, Oyesika OO. A Textbook of medicinal plants from Nigeria. Lagos:University of Lagos Press.2008;628.
9. Omokhua GE. Medicinal and socio-cultural importance of *Costus afer* (Ker Grawl) in Nigeria. International Multidisciplinary Journal, Ethiopia. 2011;5(5):22.
10. Aweke G. *Costus afer* (Ker Gawl). In: Schmelzer, G.H. and Gurib-Fakim, A. (Eds). Medicinal plants.Plant Resources of Tropical Africa. 2007;11:1.
11. Oliver BB. Medicinal plants in tropical West Africa. Cambridge: Cambridge University Press. 1986;198-199.
12. Anusha MM, Krishna N, Ranganayakulu D, Gopi SK, Vijay KG. Anti-atherosclerotic activity of the ethanol extract of the *Buteamonosperma* leaves as alone and in combination with atorvastatin. Journal of Advances in Drug Research. 2010;1:1.
13. Ahmed SI, Sumbul S. Antihyperlipidemic activity of *Carissa carandas* (Auct.) leaves extract in egg yolk induced hyperlipidemicrats. Journal of Basic & Applied Sciences. 2012;8:1.
14. Yakubu MT, Akanji MA, Oladiji AT. Aphrodisiac potentials of aqueous extract of *Fadogia agrestis* (Schweinf. Ex Heirn) stem in male albino rats. Asian Journal of Andrology. 2005;7(4):399-404.
15. Holme DJ, Peck H. Analytical biochemistry, 3rd Edn. New York: Longman.1998;350.
16. Albert B. Molecular biology of the cell. New York: Garland Science. 2002;760.
17. Rasekh HR, Nazari P, Kamil-Nejad N, Hosseinzaden L. Acute and subchronic toxicity of *Galega officinalis* in rats. J Ethnopharmacol. 2008;116:21–26.
18. Dhawan BN, Patnaik GK. Investigation on some new cardioactive glycosides. Indian Drug. 1985;2(6):285-290.

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