EFFECTS OF HYDROMETHANOLIC STEM EXTRACT OF *COSTUS AFER* KER GAWL. (COSTACEAE) ON HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN RATS

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ABSTRACT

Introduction: *Costus afer* is commonly used as a medicinal plant throughout tropical Africa. Some of the ethnomedicinal uses of the plant include treatment of malaria, diabetes mellitus, arthritis, venereal diseases, skin eruption and inflammation.

Aim: The study investigated the effect of sub-chronic oral administration of *Costus afer* stem extract on haematological and biochemical parameters in Wistar rats.

Methodology: Forty albino wistar rats of either sex (160-220 g) were randomly divided into 4 groups of 10 rats each. Group I (control) rats received 1 ml/kg distilled water orally and Groups II, III and IV rats received 250, 500 and 1000 mg/kg orally of the extract respectively daily for 28 days. The rats were sacrificed on the 29th day and blood samples were collected for haematological and biochemical analysis. An automated haematological machine (Cell-Dyn TM Abbot, U.S.A) was used to evaluate the haematological parameters and the serum lipids (Total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol) were estimated using reagent kits (Randox Laboratories Ltd, U.K) and a Colorimeter (Mediguard, India, model 110).

Results: The extract produced insignificant increases ($p>0.05$) in white blood cell count, red blood cell count and haemoglobin concentrations and insignificant ($p>0.05$) decreases in platelet count when compared with the control group (distilled water). Triglycerides and LDL-cholesterol were decreased but total cholesterol and HDL-cholesterol were significantly ($p\leq0.05$) elevated.

Conclusion: The results showed that hydromethanolic stem extract of *Costus afer* has no significant effect on haematological parameters of Wistar rats at the doses tested, but it showed some hypolipidemic effects that may be useful in reducing the risk of cardiovascular diseases.

Keywords: *Costus afer*, Haematological parameters, Serum lipid profile, Wistar rats

INTRODUCTION

There is an increasing use of herbal medicines globally for the management of diseases because of their efficacy, availability and affordability and it is estimated that about 75% of the world's population rely on herbs to meet their healthcare needs[1]. This has also been attributed to preference of consumers for natural therapies, erroneous belief that herbal products are superior to manufactured products, dissatisfaction with the results from orthodox pharmaceuticals and the belief that herbal medicines may be effective in the treatment of certain diseases where conventional therapies and medicines have proven to be ineffective, high cost and side effects of most modern drugs, improvements in the quality, efficacy and safety of herbal medicines with the development of science and technology and a movement towards self medication[2]. Herbal medicines, like the conventional drugs, are supposed to have product license based on safety, quality and efficacy[3], but this is not the case in many parts of the world and

especially in developing countries where many unregistered and poorly regulated herbal products are sold freely in the market with little or no restraint[3] and this poses great danger to the health of consumers of such products. Contrary to the general perception of herbal medicines being very safe and devoid of adverse effects, a number of toxicological studies have shown otherwise. Examples of adverse effects of herbal medicines that have been documented include: cardiovascular and central nervous system adverse effects of *Ephedra sinica*[4-7], hepatotoxicity of total alkaloids and senkirkine isolated from *Tussilago farfara*[8], burning sensation in the gastrointestinal tract, nausea, diaphoresis and light headedness of *Allum sativa*[9], and the cancer-inducing properties of *Ginkgo biloba* in experimental model[10].

Costus afer Ker Gawl., commonly known as ginger lily or bush cane belongs to the family Costaceae. It is one of the 150 species of tall, perennial, and rhizomatous herbs of the genius *Costus*[11]. It can attain a height of up to 4 m. It is commonly found in moist and shady forest belt of Senegal, South Africa, Guinea, Niger, Sierra Leone, Ghana, Cameroon and Nigeria[11,12]. In Nigeria, *Costus afer* is known as *Ireke omode* in Yoruba, *Kakizuwa* in Hausa, *Okpete* or *Okpoto* in Igboland, *Mbritem* in Efik. In Ijaw it is called *Ogbodou*, Anglophone Cameroon calls it 'Monkey sugar cane'[13]. *Costus afer* is used to treat a wide range of diseases in ethnomedicine and these diseases include: diabetes mellitus, inflammation and arthritis[14], cough, malaria, venereal diseases, skin eruptions[15], cuts and sores[16], toothache, headache with vertigo, oedema, fever[1]. Some of the pharmacological activities of *Costus afer* found in the literature include: antibacterial and *in vitro* amoebicidal activities[18], anti-inflammatory activities[19], *in vitro* antioxidant activities[20], abortificient properties[13,21], antipyretic activity[22], hypolipidemic effects[23] and hepatotoxicity[17]. In spite of the wide use of *Costus afer* in treating various diseases by traditional health practitioners and the fact that traditional medicine is mostly unregulated or poorly regulated, there is a dearth of scientific information about the toxic effects of this plant. This research was therefore conducted to evaluate the effects of sub-chronic oral administration of the hydromethanolic stem extract of *Costus afer* on haematological and biochemical parameters in Wistar rats with a view to providing a safety guide for its use in ethnomedicine.

MATERIALS AND METHODS

Materials

Plant material

Costus afer (whole plant) was collected from Kagoro Hills in Kagoro, Kaura Local Government Area of Kaduna State, Nigeria in September, 2017 and it was identified and authenticated by Mallam Musa, a Taxonomist in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen (Voucher number 01/1087) was prepared and deposited at the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria for future references.

Experimental Animals

Wistar rats of either sex weighing between 160-220 g were obtained from the Animal House facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. Nigeria. The animals were kept in clean, dry cages and maintained under ambient conditions of temperature and humidity and 12h/12h light/darkness cycle in the Animal House. Standard feed (Vital Feed, Jos) and water *ad libitum* were provided for the animals, except when fasting was necessary in the course of the study.

Ethical Approval

Ethical approval for the use and care of laboratory animals was obtained from Ahmadu Bello University Committee on Animal Use and Care (Approval Number: ABUCAUC/2018/015) and all experiments were carried out in accordance with the guidelines and principles of the Ethical Committee.

Methods

Acute Oral Toxicity Test

The oral median lethal dose (LD_{50}) of the hydromethanolic stem extract of *Costus afer* was estimated to be greater than 5000 mg/kg in an earlier study[24].

Sub-Chronic Toxicity Studies in Rats

The study was carried according to the OECD Guidelines[25] for repeated dose 28 days oral toxicity study of chemicals in rats. Forty (40) rats of both sexes (20 males and 20 females) were weighed (160g -220g) and randomized into 4 groups of 10 rats each (5 males and 5 females kept in separate cages). Group I (Control) rats were administered distilled water 1ml/kg body weight. Groups II, III and IV rats received 250, 500 and 1000 mg/kg body weight of the hydromethanolic stem extract of *Costus afer* orally daily respectively, for 28 days. The rats were allowed free access to food and water during the duration of the study and they were observed daily for general symptoms of toxicity (hyperactivity, sedation and salivation) and mortality. The rats were weighed once weekly and the average change in weight calculated. The rats were deprived of food overnight and then weighed and sacrificed on the 29th day under light halothane anaesthesia. Blood samples were then collected through cardiac puncture for haematological and biochemical analysis.

Haematological studies

A portion of blood sample from the sacrificed rats was collected in EDTA bottles for estimation of Packed Cell Volume, Red Blood Cell (RBC) count, Haemoglobin concentration (Hb), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cell (WBC) count and differentials and Platelet count using an automated haematological machine (Cell-Dyn TM Abbot, U.S.A).

Determination of Packed Cell Volume (PCV)

The PCV was determined by properly mixing the anticoagulated blood and then filling the haematocrit tube with the blood. One end of the tube was sealed using bunsen flame. The tube was then placed in the microhaematocrit centrifuge (Hawskley model no: 1675) and spun for 5 minutes. The PCV was read using the microhaematocrit reader.

Determination of haemoglobin (Hb) *concentration*

Haemoglobin was determined using the haemiglobincyanide (HICN) method. Whole blood was diluted 1 in 20 in Drabkin's solution. The RBCs were haemolyzed and the haemoglobin was oxidized by the ferricyanide to metheamoglobin which was then converted by the cyanide to stable haemiglobincyanide. Absorbance of the HICN solution was read at 540 nm using a colorimeter (Optima, Japan, model no: ac 116). The absorbance obtained was compared to that of a standard HICN solution.

Determination of total white blood cell, total red blood cell and platelet

The total WBCs, RBCs and platelet count were determined by diluting the blood with the appropriate diluting fluids (1 in 20 for WBCs, 1 in100 for RBCs and 1 in 20 for platelet). The diluted blood was then placed in a mounted counting chamber (Cell-Dyn TM Abbot, U.S.A). The WBCs and platelets were counted from the large squares of the counting chamber while the RBCs were counted from the small squares.

Differential white blood cell counts

A drop of blood was placed on one end of the slide and using a spreader a thin blood film was made. The blood film was then stained using Leishman stain. Using a microscope under the objective lens (x100), the dry stained blood film was examined and 100 white blood cells were counted, out of which the number of neutrophil, lymphocyte, monocyte, basophil and eosinophil were noted and recorded.

Biochemical studies

Another portion of blood samples was collected from the sacrificed rats into plain bottles, allowed to clot and centrifuged (Biobase, China, Model No: C200) at 3,500 rpm for 10 minutes. The separated sera were stored at - 4ºc, and used for the evaluation of serum lipid profile (total cholesterol, triglycerides, high density lipoprotein (HDL)-cholesterol and low density lipoprotein (LDL)-cholesterol) using reagent kits from Randox Laboratories Limited (U.K) and a Colorimeter (Mediguard, China, Model 110)

STATISTICALANALYSIS

Quantitative data were expressed as mean ± S.E.M and presented as Tables. Data were analysed using One Way Analysis of Variance (ANOVA) followed by Bonferroni post hoc multiple comparisons test. Significant differences between means were assessed at 95% level of significance i.e p-values less than or equal to 0.05 ($p \leq 0.05$) were considered significant. SPSS Version 20 (2011) software package was used to carry out the statistical analysis of the data.

RESULTS

Sub-chronic Toxicity Studies

Effect of 28 days repeated oral administration of hydromethanolic stem extract of *Costus afer* on haematological indices of Wistar Rats

The hydromethanolic stem extract of *Costus afer* at all the doses tested did not have significant effects ($p > 0.05$) on any of the haematological indices (white blood cell count, red blood cell count, haemoglobin concentration and platelet count) after 28 days of daily treatments when compared with the values obtained in the control group that received distilled water daily for 28 days. Although the values of platelet count in all the extract treated groups were lower than that of the control group, the differences were not statistically significant ($p > 0.05$) (Table 1).

Table 1: Effect of 28 days repeated oral administration of hydromethanolic stem extract of *Costus afer* on haematological indices of Wistar rats

Treatment Group	Dose (mg/kg/day)	WBCx $10^3/\mu L$	RBCx $10^6/\mu L$	Hb g/dL	Platelet count x $10^3/\mu L$
Distilled water	1 ml/kg	3.73 ± 0.30	5.95 ± 0.05	15.12 ± 0.83	238.62 ± 30.33
HMECA	250	3.93 ± 0.37	5.92 ± 0.05	15.23 ± 0.56	189.90 ± 1.79
HMECA	500	3.27 ± 0.17	6.07 ± 0.07	15.53 ± 0.72	188.20 ± 5.57
HMECA	1000	4.03 ± 0.13	5.98 ± 0.05	15.70 ±0.71	190.03 ± 4.72

Values are means ± S.E.M, n = 10, One Way ANOVA, $p > 0.05$ = No significant difference between test groups and negative control, HMECA = Hydromethanolic stem extract of *Costus afer*.

Effect of 28 days repeated oral administration of hydromethanolic stem extract of Costus afer on white blood cells and differentials in Wistar rats

The hydromethanolic stem extract of *Costus afer* at all the doses tested produced lower lymphocyte count when compared with the control (distilled water) but this decrease was only significant ($p \leq 0.05$) at the 1000 mg/kg dose (Table 2). There were dose dependent increases in monocyte/basophil/eiosinophil counts at the extract doses of 500 and 1000 mg/kg when compared with the control. However, this increase was significant ($p \leq 0.05$) only at the higher dose of 1000 mg/kg of the extract (Table 2).

Table 2: Effect of 28 days repeated oral administration of the hydromethanolic stem extract of *Costus afer* on white blood cells and differentials in Wistar rats f *Costus afer*.

Treatment Group(mg/kg/day)	WBC x$10^3/\mu l$	Lymphocyte x$10^3/\mu L$	Monocyte/ Basophil/ Eosinophil count x $10^3/\mu L$	Neutrophil count x $10^3/\mu L$
D.W 1ml/kg	3.73 ± 0.30	6.03 ± 0.20	0.32 ± 0.03	2.07 ± 0.16
HMECA 250	3.93 ± 0.37	5.70 ± 0.13^{ac}	0.32 ± 0.03^{ac}	2.52 ± 0.34
HMECA 500	3.27 ± 0.17	5.87 ± 0.08^{bc}	0.38 ± 0.04	1.90 ± 0.06^{bc}
HMECA 1000	4.03 ± 0.13	$5.02 \pm 0.07^{*abc}$	$0.45 \pm 0.03^{*ac}$	$2.93 \pm 0.33^{*bc}$

Values are means ± S.E.M, n = 10, One Way ANOVA + Bonferroni post hoc test $^{*}p \leq 0.05$ = significant difference between test groups and negative control (Distilled water) and common alphabets in superscripts = significant difference between treatment (hydromethanolic extract doses) groups at $p \leq 0.05$. HMECA = Hydromethanolic stem extract of *Costus afer*

The effect of the extract at all the doses tested on neutrophil was not in a clear pattern. 500 mg/kg dose of the extract had a lower neutrophil count than the control and although 250 and 1000 mg/kg doses of the extract had higher neutrophil counts than the control, this was only significant ($p \leq 0.05$) at the higher dose of 1000 mg/kg (Table 2).

Effect of 28 days repeated oral administration of the hydromethanolic stem extract of Costus afer on red blood cells in Wistar rats

The hydromethanolic stem extract of *Costus afer* at all the doses tested the doses did not show any significant effect ($p > 0.05$) on any of the red blood cell parameters when compared with the control (distilled water) (Table 3).

Table 3: Effect of 28 days repeated oral administration of the hydromethanolic stem extract of *Costus afer* on red blood cell parameters in Wistar rats

RBC parameter	D.W (1 ml/kg/day)	HMECA(250 mg/kg/day)	HMECA(500 mg/kg/day)	HMECA(1000 mg/kg/day)
RBC count (x 10^6/μL)	5.95 ± 0.05	5.92 ± 0.05	6.07 ± 0.07	5.98 ± 0.05
Hb (g/dL)	15.12 ± 0.83	15.23 ± 0.56	15.53 ± 0.72	15.70 ± 0.71
PCV (%)	45.33 ± 2.47	45.67 ± 1.58	47.17 ± 2.09	46.83 ± 2.23
MCV (fL)	84.93 ± 8.48	88.35 ± 7.08	88.27 ± 2.48	84.17 ± 3.52
MCH (pg)	32.52 ± 1.59	30.77 ± 1.78	34.95 ± 1.30	31.45 ± 0.88
MCHC (g/dL)	35.17 ± 1.10	35.35 ± 1.30	39.83 ± 0.42	37.67 ± 1.99

Values are means ± S.E.M, n = 10, One Way ANOVA, $p > 0.05$ = No significant difference between test and control groups for all parameters. Hb = Haemoglobin concentration, PCV = Packed cell volume, MCV = Mean cell volume, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration

Effect of 28 days repeated oral administration of hydromethanolic stem extract of Costus afer on platelets in Wistar rats

The hydromethanolic stem extract of *Costus afer* at all the doses tested decreased platelet count but not in a dose-dependent way and the decreases were not statistically significant ($p>0.05$). There were also no significant differences ($p > 0.05$) between the MPV and PCT of the extract treated groups and the control (Table 4).

Table 4: Effect of 28 days repeated oral administration of hydromethanolic stem extract of *Costus afer* on platelets in Wistar rats

Platelet parameter	Distilled water 1 ml/kg/day	HMECA 250 mg/kg/day	HMECA 500 mg/kg/day	HMECA 1000 mg/kg/day
PLT (x10^3/μL)	238.62 ± 30.2	189.90 ± 1.79	188.20 ± 5.57	190.03 ± 4.72
MPV (fL)	8.57 ± 0.23	9.81 ± 0.68	9.73 ± 0.63	8.82 ± 0.07
PCT (%)	0.13 ± 0.02	0.16 ± 0.03	0.15 ± 0.02	0.15 ± 0.02

Values are means ± S.E.M, n = 10, One Way ANOVA, $p > 0.05$ = No significant difference between test and control groups for all parameters. HMECA = Hydromethanolic stem extract of *Costus afer,* PLT = Platelet count, MPV = Mean platelet volume, PCT = Platelet concentration

Changes in serum lipid profile of rats pre-treated with hydromethanolic stem extract of *Costus afer* for 28 days

There was a general decrease in serum triglyceride levels in the extract treated groups at doses of 500 and 1000 mg/kg, but only 500 mg/kg showed significant ($p \leq 0.05$) reduction when compared with the control (distilled water). The 250 mg/kg dose of the extract increased serum triglyceride level but the increase was not significant ($p > 0.05$) when compared with the control. Bonferroni post hoc test showed significantly lower levels of serum triglycerides at 500 and 1000 mg/kg doses as compared with the 250 mg/kg dose (Table 5).

Table 5: Changes in serum lipid profile of rats pre-treated with hydromethanolic stem extract of *Costus afer* for 28 Days

Lipid indices	D.W(1 ml/kg)	HMECA(250 mg/kg/day)	HMECA(500 mg/kg/day)	HMECA(1000 mg/kg/day)
Triglycerides (mg/dL)	59.50 ± 6.74	69.33 ± 7.51^{abc}	37.67 ± 3.90^{*ab}	41.50 ± 7.37^{ac}
Total cholesterol (mg/dL)	91.42 ± 8.21	114.01 ± 8.23	127.8 ± 11.99*	129.9 ± 16.50*
HDL-cholesterol (mg/d/L)	28.17± 16.02	77.67 ± 9.12*	62.33 ± 12.32*	76.50 ± 5.21*
LDL-cholesterol (mg/dL)	59.94 ± 13.07	52.38 ± 6.80	56.67 ± 16.69	47.14 ± 13.64

Values are means ± S.E.M, n = 10, One Way ANOVA + Bonferroni post hoc test, $^{*}p \leq 0.05$ = significant difference between test groups and control and common alphabets in superscripts = significant difference between treatment groups at $p \leq 0.05$. HMECA = Hydromethanolic stem extract of *Costus afer,* HDL = High Density Lipoprotein –cholesterol, LDL = Low Density Lipoprotein – cholesterol

There were increases in serum total cholesterol levels at all the doses of the extract tested, but the increases were only significant ($p \leq 0.05$) at the higher doses of 500 and 1000 mg/kg when compared with control. Bonferroni post hoc test showed no significant differences ($p > 0.05$) in total cholesterol levels among the extract treated groups (Table 5).

The hydromethanolic stem extract of *Costus afer* at all the doses tested significantly ($p \leq 0.05$) increased HDL- cholesterol serum levels, but not in a dose dependent way, when compared with the control. There were no significant differences ($p > 0.05$) in the serum HDL-cholesterol levels among the doses of the extract tested (Table 5).

The hydromethanolic stem extract of *Costus afer* at all the doses tested insignificantly ($p>0.05$) reduced serum LDL- cholesterol levels when compared to the control (Table 5).

DISCUSSION

The effect of the hydromethanolic stem extract of *Costus afer* afer 28 days repeated oral administration in Wistar rats showed increases, even though not significant ($p> 0.05$), in WBCs, RBCs and Hb concentration. This result suggests that the hyromethanolic stem extract of *Costus afer* at the tested doses did not affect erythropoiesis, morphology or osmotic fragility of the red blood cells. The increase in some white blood cells (neutrophils and monocytes which were all significant at the 1000 mg/kg) in extract treated groups could be attributed to the defence system of the rats to provide protection from the effect of the extract[26]. All the three doses of the extract tested resulted in reduction in platelet count when compared to the control (distilled water) after 28 days oral treatment in rats. Although these decreases in platelet count were not significant ($p> 0.05$), they may be as a result of inhibition of platelet production as a result of decreased

secretion of thrombopoetin[2]. The result of this study is similar to that reported for *Hymenocardia acida leaf* extract in Wistar rats at 25, 50 and 100 mg/kg doses tested[27] which produced no significant effects on haematological parameters[27]. In another study, *Salvadora persica* extract was reported to alleviate the adverse effects on WBCs, RBCs and platelet count caused by stress in rats and the result of the stress/extract treated group was comparable to that of the control (distilled water) group[28]. Unlike the results of this extract on haematological parameters, the aqueous leaf extract of *Ocimum gratissium* produced significant ($p \leq 0.05$) decreases in the levels of haemoglobin, packed cell volume, RBCs and WBCs in rabbits[29]. The result of this experiment has shown that the *Costus afer* stem extract has no adverse effects on blood at the doses tested.

An evaluation of the serum lipid profile in Wistar rats after 28 days repeated oral administration of hydromethanolic stem extract of *Costus afer* showed reduced serum levels of triglycerides and low density lipoprotein-cholesterol, but total cholesterol and high density lipoprotein-cholesterol were elevated. The increase in total cholesterol may be due to the significant increase in high density lipoprotein-cholesterol at all extract doses tested. High density lipoprotein-cholesterol plays a protective role against cardiovascular diseases[30] principally by promoting reverse transport of cholesterol by scavenging excess cholesterol from peripheral tissues and subsequently causing the esterification of the cholesterol using lecithin cholesterol acyltransferase and delivering them to the liver and other steroidogenic organs for biosynthesis and eventual excretion[31,32]. In one study *Costus afer* stem was reported to contain about 15.55% crude fiber[33]. Fiber is known to decrease low density lipoprotein-cholesterol by interrupting cholesterol and bile acid absorption and increasing LDL receptor activity[34] and LDL is known to facilitate transport of cholesterol into cells[35]. The observed reduction in serum levels of LDL by hydromethanolic stem extract of *Costus afer* may be important in reducing the risk of cardiovascular diseases in humans. The result of this study is similar to the one obtained in ethanol-induced rat models of liver cirrhosis where they reported significant decrease in serum Triglyceride (TG), Total Cholesterol (TC), Low Density Lipoprotein (LDL)-Cholesterol, and Total Bilurubin but High Density Lipoprotein (HDL)-Cholesterol serum levels were elevated in the rats co-treated with *Costus afer* stem extract indicating a greater level of hepatoprotection in these models and *Costus afer* may be effective in the modulation of hyperlipidemia[23]. These effects may be as a result of the antioxidant properties of saponin and tannins present in the extract[23]. Dyslipidemia is a major risk factor in cardiovascular disease[36] and it is characterized by increased blood levels of low density lipoprotein-cholesterol, total cholesterol and triglycerides. In contrast, a low level of high density cholesterol is a risk factor for mortality from cardiovascular disease[37].

CONCLUSION

The results of this study have shown that sub-chronic oral administration of hydromethanolic stem extract of *Costus afer* has no adverse effects on haematological parameters in Wistar rats but it produced some hypolipidemic effects that could make the plant useful in reducing the risk of cardiovascular diseases.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

AUTHORS' CONTRIBUTIONS

Jimoh AA initiated the research, Jimoh AA and Maiha BB designed the experiments, Jimoh AA carried out the experiments under the supervision of Maiha BB, Chindo BA and Ejiofor JI, Jimoh AA wrote the draft manuscript which was reviewed by Maiha BB, Chindo BA and Ejiofor, JI. All the authors read and approved the final manuscript.

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