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RESEARCH ARTICLE

EFFECT OF COMBINED ETHANOLIC LEAF EXTRACT OF *COSTUS AFER* AND *CLEOME RUTIDOSPERMA* ON SOME BIOCHEMICAL PARAMETERS AND LIPID PEROXIDATION IN WISTAR RATS

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Abstract

The effect of combined ethanolic leaf extract of *Costus afer* (CA) and *Cleome rutidosperma* (CR) orally administered at two doses (50mg/kg and 100mg/kg) on some biochemical parameters and lipid peroxidation on rats weighing between 180-260g was evaluated. The twelve rats used for the experiment were of the wistar strain and were divided into three groups of four rats each ; group 1(control) received standard feed and water, while tests groups 2 and 3 in addition to standard feed and water received 50mg/kg and 100mg/kg body weight of the extract respectively once daily for 28 days. Phytochemical analysis carried out on the plants revealed the presence of alkaloids and flavonoids which are known to possess antioxidant properties. Tannins, saponins and glycoside were also present in the plants. All biochemical parameters (AST, ALT, ALP, total protein, albumin, total and conjugated bilirubin) analysed were not significantly different ($p>0.05$) from the control. It was thus concluded that the chemical constituents of the studied plants had no adverse effect on hepatic function and might have the potential of combating free radical as suggested by increasing levels of SOD (from 5.3±0.02 to 7.5±0.01), but decreasing MDA (7.97×10^{2}±4.15 to 7.46×10^{2}±1.0) levels. This results also suggests that peroxidation of lipids might have been reduced, as a result of superoxide dismutase which increased following extract administration

Keywords: *Costus afer*, *Cleome rutidosperma* biochemical parameters, lipid peroxidation

Introduction

Medicinal plants are of particular importance because they contain useful secondary products with high potency in the management of human ailments. it is generally assumed that the active constituents contributing to the efficacy of the medicinal plants are the phytochemicals, minerals and vitamins. Plants have provided mankind with herbal remedies for many diseases for many centuries till date. They continue to play a major role in primary healthcare as therapeutic remedies in developing countries. The role of plants in folklore medicine is attributed to the presence of phytochemicals; which are non nutritive plant chemicals that have disease preventing or curative properties.(Arhoghro *et al*,2014).

C. afer, which is commonly called bush sugar cane or monkey sugar cane (Nyananyo, 2006). *C. afer* which belongs to the family *Zingiberaceae* is a monocot and a relatively tall, herbaceous, unbranched tropical plant with creeping rhizome. It is commonly found in moist or shady forest of West

and Tropical Africa (Iwu, 2009). *C. afer* is a useful medicinal plant that is highly valued for its anti-diabetic, anti-inflammatory and anti-anthritic properties in South-East and South-West Nigeria (Soladoye and Oyesika, 2008).

Cleome rutidosperma grows principally at low altitudes in humid conditions *Cleome rutidospermalinn*(family-cleomaceae) is use to relieve earache, pain, skin disease. In Ghana, Gabon and DR Congo leaf sap is applied to cure earache and deafness. The plant is used in the treatment of paralysis epilepsy, convulsion and spasm (Anuradha, *et al*., 2013*).*

According to Tiwari and Rao (2002) polyherbal therapies have the synergistic, potentiative, agonistic/antagonistic pharmacological agents within themselves, that work together in a dynamic way to produce therapeutic efficacy with minimum side effects. It is in this light that this work was designed to investigate the effects of combined extracts from two widely used plants *Costus afer* and *Cleome rutidosperma* employed traditionally in the management of some maladies.

This study evaluated the effect of combined ethanolic Leaf extract of *Costus afer* and *Cleome rutidosperma* on some biochemical parameters and lipid peroxidation in Wistar rats.

Materials and Methods

Animals

Twelve (12) adult healthy male albino rats, weighing between 180 and 260g were used in this study. The rats were obtained from the animal house of the Niger Delta University, College of Health Sciences, Bayelsa State and housed in standard cages. They were then allowed free access to standard feed (growers mash) and water for a period of two weeks to acclimatize to the cage environment prior to the commencement of the experiment. All the protocols were performed in accordance with the Institutional Animal Ethical committee (IAEC) as per the directions of the committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Chemicals

All chemicals used were of analytical standard

Preparation of extracts

Fresh leaf of *costus afer* and *cleome rutidosperma* were collected within Amassoma, Yenagoa, Bayelsa State. collected from a residential farmyard in Niger Delta University (N.D.U),Amassoma, Wilberforce Island, Bayelsa State, Nigeria and was botanically identified and deposited at the Herbarium of department of biological science, in Niger Delta University (N.D.U), Amassoma, Wilberforce Island, Bayelsa State, Nigeria. These were washed with distilled water, shade dried and pulverized. The leaves of *Costus afer* and *Cleome rutidosperma* were thoroughly washed with distilled water to remove debris and contaminants, they were then dried in an oven at 40℃ until a constant weight was reached, and then pulverized using an electric blender (Blender, 462 Nakai Japan). 200g of the powdered mixture (i.e 100g each of *Costus afer* and *Cleome rutidosperma*) was extracted in 600ml of absolute ethanol for 24 hours at room temperature with constant shaking using a flask shaker (Model, Denly A-500). The extract was filtered with Whatman No 1 filter paper and the resulting filtrate evaporated to dryness using a rotatory evaporator at 40 ℃ , the resultant concentrate was then reconstituted in distilled water to give the required doses used in the study.

Phytochemical screening

Phytochemical tests were conducted on the sample of leaves of *Costus afer* and *Cleome rutidosperma* to determine the presence of alkaloids, anthraquinone, tannins, terpenoids, saponins, flavonoids, cardiac glycosides and carbohydrates using standard protocols (Sofowora, 1993; Trease and Evans, 1993).

Experimental design and procedures

Experimental design

Twelve rats of the wistar strain weighing between 180-260g were distributed into three groups of four (4) animals each.

Group 1 served as the normal control group and received standard feed and water, while group 2 and 3 were the test groups and they received 50mg/kg body weight and 100mg/kg body weight of the combined leaf extract respectively.

Biochemical Analysis

Sample Collection

After the experimental period, animals in different groups were sacrificed. By the end of each experimental period, the rats were reweighed, starved for 24 hours and sacrificed under chloroform anesthesia. 5ml of blood was collected from each animal by cardiac puncture using sterile needle and syringe. Part of the blood sample was put into test tubes and allowed to clot for 30 minutes before centrifuging at 800g for 5 minutes. The supernatant was used for the biochemical analysis.

Preparation of liver Homogenate

The livers were also excised and washed in cold saline. Ten percent tissue homogenates were prepared in 0.1M Tris -HCl buffer (pH 7.4). Perinuclear fractions were obtained after centrifuging homogenates at 1500 rpm for 20 minutes using a centrifuge.

Biochemical estimation

The following liver function test were conducted to investigate derangement in the liver of the animals used for the study, aspartate aminotransferase (AST), alanine aminotransferase (ALT) were determined by the colorimetric method of Reitman and Frankel, 1957 using a commercial assay kit from Randox Laboratories Ltd, Co. Antrim, United Kingdom. Alkaline phosphatase (ALP) was extimated by the colorimetric method of REC, 1972 using assay kits from Randox Laboratories Ltd. Serum protein and serum albumin were estimated by Biuret method and Bromocresol Green (BCG) binding method respectively using a commercial assay kit from Randox Laboratories Ltd.

Total and conjugated bilirubin was determined using commercial kits from Randox Laboratories Ltd, using colorimetric method described by Jendrassik and Grof, 1938

Markers of oxidative disturbances

Super oxide dismutase (SOD) activity was by the methods of Misra and Fridovich (1972). The assay method of Hunter *et al*. (1963) as modified by Gutteridge and Wilkins (1980) was adopted for the assay of Malondialdehyde (MDA) concentration.

Statistical Analysis

The result obtained from the study were analysed using the statistical package for social science (spss) version 16.0 for windows. One-way ANOVA followed by Post-hoc Turkey was used to compare mean ± S.D, and values were considered significant at $p< 0.05$.

Results

Phytochemcial compounds are known to have beneficial importance in industrial and medicinal sciences. Qualitative analyses of both plants revealed the abundance of alkaloids and flavonoids (++). Found also in abundance in *C.rutidosperma* are tannins and saponins, while glycoside was present in trace (+). Saponins, tannins and glycoside were present in trace (+) in *C.afer.*

Proximate composition (Table 2) of the plant *C.afer* is as follows-4.8% ash, 10.48 %protein, fibre 4.6, lipid content 2.30 and moisture content 1.86 whereas that of *C.rutidosperma* is as follows-%ash 5.20, %protein 11.68, lipid content 2.7, fibre 5.40 and moisture content 2.20.

Weights of animals for test groups 2 (from 242.5±11.0 to 259.8±21.0) and 3(from 181.33±30.3 to 249.21±19.9) were found to increase following extract administration of 50mg/kg and 100mg/kg body weight. of the extract had the highest weight gain. (fig 1).

There was slight increase in total protein levels (Table 3) from 62.7±1.07 to 72.8±1.28($p>0.05$), and the activity of serum enzymes – ALT and AST from 20.1±1.52 to 21.5±1.2 and 18.3±0.9 to 20.7±1.42 respectively. ALP levels decreased ($p>0.05$) from 29.5±1.29 ($p>0.05$) following extract administration. Albumin was negligibly decreased ($p>0.05$) from 24.5±1.29 to 24.3±2.95 ($p>0.05$) from 7.50±1.29 to 8.27±1.10 (Table 3)

There was decreased MDA levels from 7.97 $\times 10^2 \pm 4.15$ to 7.46 $\times 10^2 \pm 1.0$ ($p<0.05$), while SOD increased ($p<0.05$) across the group, with group 1 having a value of 5.3±0.01, while groups 2 and 3 had values of 7.1±0.01 and 7.5±0.01 respectively.(Table 4)

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Discussion

The presence of phytochemicals in plant species has contributed greatly to ethnomedicine in many

Table 1. Phytochemical constituents of leaves of *Costus afer* and *Cleome rutidosperma*

Phytochemicals	*Costus afer*	*Cleome rutidosperma*
Alkaloids	++	++
Tannins	++	++
Saponins	+	++
Flavonoids	++	++
Glycosides	+	+

Key; +trace; ++abundance

Table 2. Proximate Composition of leaves of *Costus afer* and *Cleome rutidosperma*

Biological compounds	*Costus afer*	*Cleome rutidosperma*
Ash (%)	4.8	5.20
Protein (%)	10.48	11.68
lipid (%)	2.30	2.70
Fibre (%)	4.6	5.40
Moisture content (%)	1.86	2.20

Fig 1: Animal weights before and After Extract Administration of Combined Ethanolic Leaf Extract of C.*afer* and C.*rutidosperma*

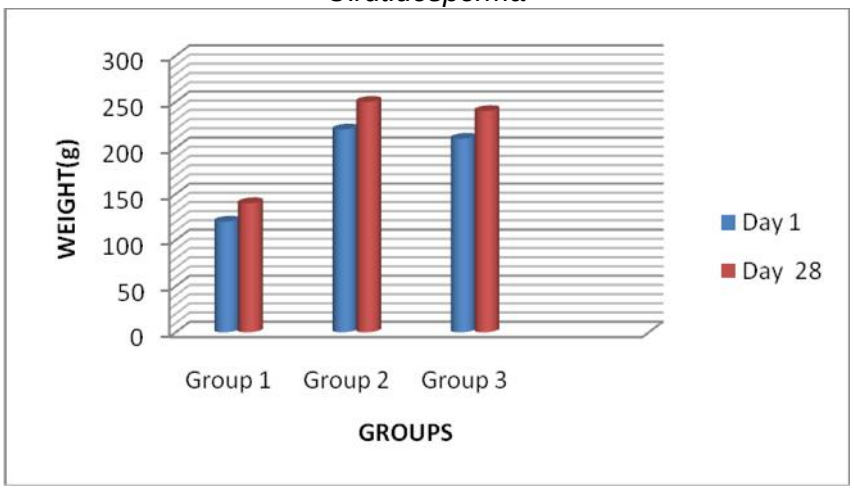

Table 3. Effects of Combined Ethanolic Leaf Extract of C.*afer* and C.*rutidosperma* on Some Biochemical Parameters

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Albumin (g/dl)	Total protein (g/dl)	Conjugated bilirubin (umol/L)	Total bilirubin (umol/L)
1(control)	20.2± 1.52^a	18.3± 0.79^a	29.5± 1.29^a	24.4± 1.29^a	62.7± 1.07^a	1.35± 0.13^a	7.50± 1.29^a
2(50mg/kg body weight)	21.1± 2.60^a	20.4± 2.16^a	28.3± 2.51^a	25.0± 2.58^a	68.2± 0.83^a	1.27± 0.17^a	7.45± 1.57^a
3(100mg/kg body weight)	21.5± 1.29^a	20.7± 1.42^a	27.9± 8.3^a	24.3± 2.75^a	72.8± 1.28^a	1.25± 0.20^a	8.27± 1.10^a

Data mean±SD (n = 4). Mean in the same column with different superscript letter(s) are significantly different $p<0.05$ (one way ANOVA followed by Post-hoc Turkey)

Table 4. The Effect of combined Ethanolic leaf Extract of C.*afer* and C.*rutidosperma* on Superoxide Dismutase Activity and Malondialdehyde Levels in rats

Groups	SOD activity (U/mg protein)	Malondialdehyde (nmol/g liver tissue)
1(control)	5.3 ± 0.02^{a}	$7.97\times10^{2}\pm4.15^{a}$
2(50mg/kg body weight)	7.1 ± 0.01^{b}	$7.64\times10^{2}\pm1.78^{b}$
3(100mg/kg body weight	7.5 ± 0.01^{c}	$7.46\times10^{2}\pm1.0^{c}$

Data mean±SD (n = 4). Mean in the same column with different superscript letter(s) are significantly different p<0.05 (one way ANOVA followed by Post-hoc Turkey)

developing countries. Phytochemical compounds are known to be beneficial in both industrial and medicinal sciences. Qualitative analyses of both plants showed abundance (++) of alkaloids and flavonoids (Table 1) which have free-radical scavenging ability by the reason of their antioxidant activity (Aiyelaagbe and paul, 2009). *Cleome rutidosperma* showed the presence of glycoside (+), and the abundance of tannins, saponins and glycoside. Analysis of *Costus afer* revealed the presence of tannins, saponins and glycoside. The finding is in agreement with the study of Akpan *et al.,* (2012) who showed *Costus afer* to possess alkaloids, flavonoids, tannins, saponins, steroids and glycosides. The plausible effect of extract on biochemical parameters evaluated and the observed decrease in MDA levels and increase in SOD (p<0.05) might be due to the presence of alkaloids and flavonoids in plants which have been severally reported to possess antioxidant properties, thus the extract might contain potent substances that are capable of combating free radicals.

Group 3 which was administered 100mg/kg body weight of extract had the highest weight gain (fig 1). This was followed by group 1(16.23%) and group 2(4%). Weight gain might be due to the anti-depressant activities of *C.afer* and *C.rutidosperma* (Anyasor *et al.,* 2010: Bose *et al.,* 2012).

Hepatic injury resulting from the active constituents of medicinal plants and failure of the liver to eliminate these metabolites often results in marked distortion of the normal functioning of the liver (Kpomah *et al.,* 2012). Elevation of certain liver enzymes aids diagnosis-AST and ALT for instances are notably elevated in hepatocellular damage, while increase in ALP might be due to intrahepatic obstruction (Madukosiri, 2013). Increase in serum enzymes AST and ALT activities, and decrease in ALP (Table 3) is in line with the study conducted by Madukosiri (2013). However, in the present study elevation of these enzymes were not suggestive of hepatocellular damage as differences between treated and control groups were not significant (p>0.05). Albumin is essential for tissue growth and aids in preventing the leakage of fluids from blood vessels. It plays an important role in transporting both endogenous and exogenous substances, serving as protein reserves, as well as maintaining osmotic pressure (Kpomah *et al.,* 2012). After 28 days of extract administration, albumin and total protein concentrations were not significantly altered (p>0.05) when compared to the control group (Table 3). This finding is in agreement with studies conducted by Anofi *et al.,(*2012) who evaluated the toxicity profile of *A.indica* stem bark in male wistar rats and Adebayo *et al.,* (2003) who studied the effect of ethanolic extract of *K.senegalensis* on some biochemical parameters of rat kidney. These studies showed that the extract of plants had no significant alterations of albumin and total protein levels, thus the liver was not impaired and as such was able to carry out its normal metabolic functions.

Bilirubin is formed from the breakdown of haemoglobin in the liver, spleen and bone marrow and its measurement is an important index in determining the excretory function of the liver and assessment of haemolytic anaemia (Kpomah *et al.,* 2012). Both conjugated and total bilirubin for test groups were not significantly different (p>0.05) from the control group, indicating that the active constituents of the plants had no adverse effect on haemoglobin metabolism and liver function of treated rats.

The peroxidation of lipids has been linked to several pathological disorders. Oxidation of lipid molecules causes damage to tissue resulting to the development of several physiological and pathological disorders (Chaturvedi, 2007). From the result obtained (Table 4), the antioxidant effect of

the plant is further suggested by the decrease ($p<0.05$) in MDA levels (a product of lipid peroxidation) and increase ($p<0.05$) in SOD activity. This result agrees with the findings of Chaturvedi (2007), on *Raphanus sativus.* The result from that study showed that the extract increased the level of reduced glutathione and catalase activity in the liver and plasma of treated rats, and these changes were significant ($p<0.05$) when compared to the control. Increase in SOD activity could lead to increase total antioxidant status of the body of animals (Valko *et al.,* 2007).

In conclusion, the chemical constituents of the studied plants had no adverse effect on hepatic function and might have the potential of combating free radical as suggested by increasing levels of SOD (from 5.3±0.02 to 7.5±0.01), but decreasing MDA ($7.97\times10^2\pm4.15$ to $7.46\times10^2\pm1.0$) levels. This results also suggests that peroxidation of lipids might have been reduced, as a result of superoxide dismutase which increased following extract administration.

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