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Original Research Article

Antihyperlipidemic and Antioxidant Activity of *Syzygium Aromaticum* Extract in Rats Fed Cycas Diet

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ABSTRACT

Cycads contain toxins, when consumed may cause oxidative stress, hepatotoxicity and even colon cancer. Spices show potential health benefits as they possess hepatoprotective, antihyperlipidemic and antioxidant activities. Hepatotoxicity chemoprevention by using antioxidant approaches has been suggested to offer a good potential of central importance. The study was aimed at ascertaining the effect of *Syzygium aromaticum* (clove) on lipid profile and antioxidant status of rat fed cycas formulated diet. Twenty albino Wistar rats (100 - 180 g) were used. The rats were fed normal diet only (Group 1), cycas diet only (Group 2), cycas containing diet plus *S. aromaticum* extract (Group 3), and normal diet plus *S. aromaticum* extract (Group 4). The aqueous extract of *S. aromaticum* was administered orally at 200 mg/kg b.w and the diets were fed for 28 days. The parameters evaluated for antioxidant activity were reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) while total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triacylglycerol (TAG) and malondialdehyde (MDA) were determined for lipid profile using standard methods. The results showed significantly lower SOD, CAT, GSH and HDL-C level and higher TC, TAG, LDL-C and MDA level in liver of rats fed cycas containing diet in comparison with rats fed normal diet. The administration of the extract showed significantly higher CAT and SOD activity and lower MDA level when compared with the animals fed cycas containing diet only. It is concluded that the extract protects against cycas-induced toxicity.

Keywords: Antioxidant, Clove, Cycas, Diet, Lipid profile.

Introduction

Natural products significance in both traditional and modern medicines cannot be over-emphasized. These may be due to their immense contributions to the well-being of man. Plants have been generally used worldwide for the treatment of diseases.[1] Cycad is an ancient *gymnospermous* plant that is extremely toxic to animals if ingested.[2] Consumption of cycas plant may be associated with oxidative stress, hepatotoxicity and colon cancer.[3,4] In colorectal cancer blood from the bowel flows to the liver directly. The liver is the main target of colorectal cancer to spread to.[3] Oxidative stress alterations caused by cycas may be due to increase in reactive oxygen species (ROS) and also inhibition of antioxidant systems.[3] Humans who eat spices benefit from the antioxidant properties of the spices.[5-7] Antioxidants are molecules that does oxidation impediment of other molecules.[7] However, a paramount measure is carried out by spices with antioxidant properties in regards to disease prevention.[8] Clove (*Syzygium aromaticum*) is a spice use around the world (including Nigeria in Africa). Clove bud is aromatic and can be used as stimulant for gastric irritation and dyspepsia.[8, 9] Every cell produces antioxidant enzymes called SOD, CAT and glutathione peroxidase (GPx) which may help in suppressing oxidative stress.[8] Altered activities of these enzymes and lipids were revealed to be indispensable in multi-stage carcinogenesis of both rats and humans.[10, 11]

The study was aimed to assess the effect of *S. aromaticum* aqueous extract on lipid profile and some antioxidant parameters in the blood and liver of rats fed cycas containing diet.

Materials and Methods

Cycas Plant and Spice (S. aromaticum)

Cycas plant was acquired from Abraka Delta State. *S. aromaticum* (clove) was purchased from Ekpoma main market, Edo State. The cycas plant and clove were collected during March- April, 2018. The spice and cycas plant (*Cycas revolute*) were identified at Forest Research Institute of Nigeria, Ibadan, with voucher number of FHI 110603 and FHI 6013157596 deposited in their Herbarium.

Experimental animals

Eight weeks old albino Wistar rats with weight range of 100 - 180 g was used for the study. The rats were purchased from the animal house in the Anatomy Department, Basic Medical School, Delta State University, Abraka. The animals were aclimatized for 7 days. They were housed in plastic cages with wire gauze. They had a 12h light/ dark cycle and fed with growers mash and had water *ad libitum*. Animals were maintained in accordance with the guidelines and protocol for care and standard use of laboratory animals by the Canadian Council and Faculty of Science ethical committee (with approved number, REC/FOS/20/010) of Delta State University, Abraka.

Preparation of the spice aqueous extract

Spice was dried in sun for two weeks till a constant weight was attained and then crushed with a blender into smooth powder. One hundred grams of the powdered spice was extracted with 500 ml of water (60^{o}C), and was allowed to stand for 48 hrs. A clean muslin

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cloth was used to sieve the mixture. The filtrate was concentrated to dryness using water bath (40^{o}C). One gram of *S. aromaticum* dried sample (crude extract) was reconstitution with 9 ml of distilled water.

Acute toxicity study of AESA
The acute toxicity of the extract was determined in order to select a suitable dose for the protective study. This was done using the method described by Lorke [12]. In the initial phase, rats were divided into 4 groups of 4 rats each and were treated with 100 mg, 200 mg, 500 mg and 1000 mg per kg body weight aqueous extract of *S. aromaticum* (AESA) orally. The rats were treated daily for 7 days, and then observed for signs of toxicity, including death. No mortality was observed after the administration of the extract orally to the rats at the dose of 100 to 1000 mg/kg b.wt for 7 days. Growth of some little new hair in rats given 200 mg/kg b.wt. and decrease in locomotion of rats given 500 to 1000 mg/kg b.wt AESA were some of the features observed.. However, 200 mg/kg body weight was used as a protective dose.

Preparation of cycas containing diet and normal diet
The cycas plant was sun dried for duration of two weeks and then ground to fine powder. The diets for each group (normal diet and cycas containing diet) were formulated by mixing known quantities of sources of each food class comprising: garri, soya bean, palm oil, cellulose in form of corn cob, salt mix, vitamin mix, and sucrose. The food components were purchased from Abraka local market. The vitamins and minerals mix (manufactured by Hebei Vsyong Animal (HVA) Pharmaceutical Co. Ltd, China) was also purchased in Abraka (Table 1).

Experimental protocol
Twenty (20) rats were shared into four groups each comprised of five rats and treated as follows: Group 1: Control (rats fed normal diet only). Group 2: Cycas control (cycas containing diet only). Group 3: Rats fed cycas containing diet + 200 mg/kg body weight AESA. Group 4: normal diet + 200 mg/kg body weight AESA. The administration of the extract was done orally for 4 weeks. On the final day of the experiment, the rats were subjected to fast overnight and sacrificed by cervical decapitation. Blood was collected in heparinised tubes for blood GSH determination and liver was harvested immediately. Zero point five gram (0.5 g) of tissues were homogenized in 4.5 ml of normal saline and centrifuged at 2,500 RPM for 15 minutes to obtain the supernatant which was used for the biochemical analysis.

Estimation of GSH
The GSH level in blood and tissue was estimated by Beutler *et al*. [13] and Ellman [14] method respectively. The sulfhydryl group of glutathione react with DTNB (5, 5, - dithiobisnitro benzoic acid, Elman's reagent) to produce a yellow colour, 5- thio -2- nitrobenzoic acid (TNB). Glutathione reductase reduces the mixed disulphide formed which yields more TNB and recycle the GSH. TNB produced is proportional to GSH in samples measured at 412 nm wavelength using a spectrophotometer.

Assay of SOD activity
Misra and Fridovich method[15] was utilized for the assay of SOD activity. Superoxide dismutase exhibits autoxidation of epinephrine by superoxide radical (O_2^-). The assay of SOD is an indirect method which is centred on the inhibitory effect of SOD in the initial rate of epinephrine autoxidation which is derived from the reaction proposed by Misra and Fridovich,[15] for the base catalysed autoxidation of epinephrine. The absorbance was checked at 480 nm every 30 seconds for 120 seconds.

Assay of CAT activity
Cohen *et al.* technique[16] was adopted for the assay of CAT activity. CAT breaks down H_2O_2 directly into water and oxygen. Spectrophotometer was used to examined the decrease in H_2O_2 concentration at 480 nm for 30 minutes.

Evaluation of Lipid peroxidation (LPO)
Lipid peroxidation in form of malondialdehyde (MDA) was assessed by the procedure of Buege and Aust.[17] The thiobarbituric acid (TBA) to measure LPO after reaction with the sample indicated an increase in absorbance at 532 nm.

Estimation of TC
Total cholesterol was estimated using Richmond method.[18] The enzymatic process involves a series of coupled reactions that hydrolyzes cholesteryl esters and oxidize the 3-OH group of cholesterol. Hydrogen peroxide which is a by-product of the reaction is measured quantitatively in a peroxidase catalysed reaction that produces a colour. The colour intensity is proportional to the cholesterol concentration measured at 500 nm.

Estimation of HDL-C
HDL-C was estimated using the method of Badimon *et al.* [19] In this reaction, the reagent is only for treatment of specimen before the assessment of HDL –C with a reagent for total cholesterol. High-density lipoprotein – cholesterol obtained in supernatant after centrifugation was measured with total cholesterol at absorbance of 500 nm.

Determination of LDL-C
Low density lipoprotein cholesterol was calculated using Friedewald formula as follows;

$$LDL - C\ (mg/dL) = \frac{(Total\ cholesterol\ - HDL) - Triacylglycerol}{5}$$

Determination of TAG
Triacylglycerol was assayed using method of Fossati and Prencipe,[20] TAG is hydrolyzed by lipase to form glycerol and free fatty acid. The process involves enzymatic assay together with Trinder reaction which ends when quinoneimine dye is formed. The amount of dye formed, determined by its absorption at 540 nm, was directly proportional to the concentration of TAG in the samples.

Statistical analysis
The data obtained was analysed using Analysis of Variance (ANOVA). The results were expressed as Mean ± SD. Significant difference of means were determined at 5% ($p< 0.05$) confidence level using Post hoc test (LSD; least significant difference).

Table 1: Diets compositions

Components	Normal diet (%)	Cycas containing diet (%)
White Garri	51.7	51.7
Soya bean (defatted)	20.0	20.0
Palm oil	5.0	5.0
Cellulose	5.0	5.0
Salt mix	3.0	3.0
Vitamin mix	2.3	2.3
Sucrose	13.0	3.0
Cycas	-	10

Results and Discussion

GSH level, Antioxidant enzymes and LPO level of rats
In all cycad genera, cycas is found [21] and well-known to be carcinogenic in four animal species which are rat, hamster, guinea-pig and fish; inducing tumours in various organs.[10,22] Cancer deterrence using antioxidant approach is suggested to offer important benefit to public health, clinicians and institutional researchers as a key strategy

for setback or delaying of carcinogenesis.[23,24] Animal have an effective mechanism to inhibit tissue damage caused by free radical which induces oxidative stress through the help of antioxidant enzymes and protein such as SOD, CAT, GPX and GSH.[25] Table 2 shows the GSH level in the blood and liver of cycas fed rats. There was a significantly ($p < 0.05$) lower GSH levels in liver and blood of rats fed with cycas diet only compared with rats fed the normal diet. Rats fed cycas diet and 200 mg/kg body weight of clove extract showed significant ($p < 0.05$) higher GSH level in liver and blood compared to rats fed cycas diet only. However, liver and blood GSH level were significantly ($p < 0.05$) lower in rats fed normal diet only compared to that given 200 mg/kg body weight of clove extract. Table 3 illustrates how clove extract protects antioxidant enzymes and prevent LPO in liver of rats fed cycas diet. The results indicated a significantly ($p < 0.05$) lower SOD and CAT activity as well as a higher MDA level in fed cycas diet compared with normal diet. Also, rats fed cycas diet and 200 mg/kg body weight of clove extract had a significant ($p < 0.05$) high SOD and CAT activity as well as a low MDA level in liver compared to rats fed with cycas diet only. The decreased activities of CAT, SOD and GSH level in blood and liver (Tables 2 and 3) of rats fed cycas containing diet only, may be as a results of the vital role of these antioxidant molecules against oxygen free radical stress. Also, this decrease may point to rapid exhaustion of the enzymes in fighting the free radicals generated during cycas toxicity. Other studies have shown that the altered activity of antioxidant enzymes is essential in multi-stage carcinogenesis of both rodents and humans compared to their appropriate normal cell counterparts.[10] However, antioxidant activity was increased when aqueous extract of clove was administered. The results of the study are in agreement with the results of Perše *et al.*[3] who showed that natural antioxidants have potential to inhibit cancer in animal model. An increased MDA in liver of rats fed cycas containing diet only (Table 3) may involve in the commencement of liver lesions. However, LPO changes cell membranes fluidity, increases membrane permeability and inflammation.[26] Treatment of rats given cycas containing diet with AESA decreased the level of lipid peroxidation**.** Eugenol a main constituent of *S. aromaticum* has anti-inflammatory effect which may be associated to its regulation of redox status, reduction of LPO and enhancement of antioxidant enzymes.[27]

Changes in Lipid profile of rats

After 28 days of treatment, significant ($p < 0.05$) increase in TC, TAG, LDL-C and decrease in HDL-C levels were observed in rats given cycas diet only compared with rats administered normal diet. Treatment of rats given cycas diet with 200 mg/kg body weight of clove extract showed significantly lower TC, TAG, LDL-C and higher HDL-C levels in liver when compared to rats given cycas diet only. Moreover, significant ($p < 0.05$) decrease in TC, TAG, LDL-C and increase in HDL-C were observed in liver of fed normal diet compared with rats given 200 mg/kg body weight of clove extract.

The significant elevation in TC, TAG, LDL-C levels and reduction in HDL-C levels in liver (Table 4) of rats fed cycas containing diet only compared to rats fed normal diet observed in the study collaborates with previous results of Eriyamremu *et al*. [23] who reported the early biochemical events of mice given cycas and a Nigerian like diet. The significant increase in TC and TAG level in rats of cycas control compared with normal control may be due *de novo* lipid synthesis. According to Al-Otaibi *et al*.[25] the combined effect of lipid degradation and *de novo* lipid synthesis induced by oxidative stress could be an attempt to meet lipid requirements for growth and metabolism.

Table 2: GSH of rats fed formulated diets

Groups	Blood GSH (mg% of white blood)	Liver GSH (unit/g wet tissue)
Normal diet only	9.27 ± 2.52^{a}	12.23 ± 3.43^{a}
Cycas diet only	4.29 ± 1.55^{b}	5.29 ± 1.61^{b}
Cycas diet + *S. aromaticum* extract	7.14 ± 1.47^{c}	9.66 ± 2.90^{c}
Normal diet + *S. aromaticum* extract	12.47 ± 2.96^{d}	19.53 ± 6.96^{d}

Values are given in mean ± SD., n = 5. Mean values with different superscript letter (a, b, c, d) in the same column differ significantly at $p < 0.05$.

Table 3: Liver SOD, CAT and MDA of rats

Groups	SOD (units/g wet tissue)	CAT (units/g wet tissue)	MDA(units/g wet tissue)
Normal diet only	28.33 ± 6.04^{a}	60.39 ± 15.78^{a}	3.49 ± 1.04^{a}
Cycas diet only	16.39 ± 7.61^{b}	36.18 ± 4.43^{b}	8.12 ± 2.59^{b}
Cycas diet + *S. aromaticum* extract	23.85 ± 7.84^{c}	48.16 ± 7.25^{c}	5.36 ± 2.10^{c}
Normal diet + *S. aromaticum* extract	33.20 ± 6.63^{d}	69.94 ± 8.03^{d}	0.85 ± 0.55^{d}

Values are given in mean ± SD., n = 5. Mean values with different alphabet in the same column differ significantly at $p < 0.05$.

Table 4: Liver lipid profile of rats

Groups	TC (mg/dL)	TAG (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
Normal diet only	224.60 ± 18.48^{a}	190.04 ± 11.01^{a}	32.09 ± 23.08^{a}	160.90 ± 14.01^{a}
Cycas diet only	301.28 ± 39.15^{b}	270.09 ± 17.34^{b}	129.91 ± 34.56^{b}	117.35 ± 14.91^{b}
Cycas diet + *S. aromaticum* extract	266.80 ± 14.87^{c}	246.97 ± 30.15^{c}	87.03 ± 14.35^{c}	130.36 ± 21.47^{c}
Normal diet + *S. aromaticum* extract	201.02 ± 26.56^{d}	177.47 ± 19.59^{d}	17.26 ± 12.29^{d}	179.20 ± 14.41^{d}

Values are represented in mean ± SD, n = 5. Means sharing different superscript alphabet of the same parameter differ significantly at $p < 0.05$.

The high concentration of LDL – C level in rats fed cycas containing diet could probably be due to increase in hydrolysis of cholesterylesters. Cholesterylester hydratase hydrolyses cholestrylesters and the activation of apo – B, thus increasing the level of cholesterol in liver tissue.[28]The significant ($p < 0.05$) decrease in HDL-cholesterol in rats fed cycas diet only could be associated with alteration in lipid metabolism caused by cycas induced free radicals which results in oxidative stress. This is in line with Venkatachalam *et al.*[29] who reported that azoxymethane which is a main metabolite of cycas induces free radical formation and lipids alterations. However, the administration of AESA to rats fed cycas diet enhances liver lipid profile.

Conclusion

Cycas induced oxidative stress results in alterations of biochemical parameters. The study showed that *S. aromaticum* extract demonstrated antihperlipidemic and antioxidant protective effect against oxidative stress and lipid peroxidation in the liver of rats fed cycas containing diet.

Conflict of Interests

The authors declare no conflict of interest.

Author's declaration

The authors declare that the work in this article is original and that any liability for claims relating to this article content will be borne by them.

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References

1. Deora GS, Shekhawat MK, Sarswati. Ethnobotanical, Phytochemical and Pharmacological Potential of Cycas revoluta Thunb - A review. Pharmacogn J. 2020;12(5):1165-1171.
2. Lal K, Ahmed N, Mathur A. Study of the antimicrobial profile and phytochemical composition of solvent extracts of leaves and female cones of *Cycas revoluta*. Int J Curr Microbiol Appl Sci. 2017; 6(4):2514-2522.
3. Perše M, Injac R, Djordjevic A, Štrukelj B, Cerar A. Protective effect of fullerenol nano particles on colon cancer development in dimethylhydrazine rat model. Dig J Nanomater Bios. 2011; 6(4):1535-1543.
4. Zarybnicky T, Bousova I, Ambroz M, Skalova L Hepatotoxicity of monoterpenes and sesquiterpenes. Arch Toxicol. 2018; 92:1-13.
5. Okpoghono J, George BO, Achuba FI. Assessment of antioxidant indices after incorporating crude oil contaminated catfish and African nutmeg (*Monodora myristica*) extracts into rat diet. J Appl Sci Environ Manage. 2018a; 22 (2):197-202.
6. Okpoghono J, Achuba FI, George BO. Protective effect of *Monodora myristica* extracts on crude petroleum oil-contaminated catfish (*Clarias gariepinus*) diet in rats. Int J Vet Sci Med. 2018b; 6:117-122.
7. George BO, Atalor B, Okpoghono J. Role of vitamin E, vitamin A and aqueous extract of *Aframomum sceptrum* in mice, infected with malaria parasites. Nig J Nutr.Sci. 2019; 40(1):113-117.
8. Otuaga EJ, Okpoghono J, George BO. Non-heme iron content and antioxidant activities of Wistar rats fed aqueous extract of *Elaeis guineensis* (banga soup) cooked using different utensils. J Appl Sci Environ. 2020; 24(6):1071-1077.
9. George BO and Okpoghono J. Antioxidant activity of water, ethanol and diethyl ether extracts of *Monodora myristica* and *Syzygium aromaticum*. Direct Res J Public Health Environ. Technol. 2017; 2(4):30-35.
10. Sun Y. Free radicals, antioxidants enzymes and carcinogenesis. Free Rad Biol Med. 1990; 16:275-282.
11. Zarkovic N, Cipak A, Jaganjac M, Borovic S, Zarkovic K. Pathophysiological relevance of aldehydic protein modifications. J Proteomics. 2013; 92:239-247.
12. Lorke, D. A new approach to practical acute toxicity testing, Toxicol Pathol. 1983; 54:275-287.
13. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963; 61:882 -888.
14. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82:70-77.
15. Misra HP and Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a sample assay for superoxide dismutase. J Biol Chem. 1972; 247:3170-3175.
16. Cohen G, Dembiec D, Marcus J. Measurement of catalase activity in tissue extracts. *Analyt Biochem*. 1970; 34:30-38.
17. Buege JA and Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978; 52:302-305.
18. Richmond W. Colorimetric method of determination of total cholesterol and lipoproteins. Clin Chem.1973; 19:1350-356.
19. Badimon JJ, Badimon L, Fuester V. Determination of HDL-cholesterol. J Clin Invest. 1990; 85:1234-1241.
20. Fossati P and Precipe L. Determination of triglyceride levels in rat tissue. Clin Chem. 1982; 28:2077-80.
21. Zhukov AV, Kuznetsova EI, Pchelkin VP, Sidorov RA, Tsydendambaev VD. Fatty acid composition of lipids from leaves and Strobila of *Cycas revoluta* (*Cycas revoluta*). Russ J Plant Phys. 2018; 65(1):23-29.
22. Almeida VL, Leitão A, Reina LCB, Montanari CA, Donnici CL. Cancer and cell cicle-specific and cell cicle nonspecific anticancer DNA-interactive agents: an introduction*;* Química Nova, 2005; 28(1):118-129.
23. Eriyamremu GE, Osagie VE, Alufa OT. Early Biochemical events in mice exposed to Cycas and fed a Nigerian like diet. Ann Nutr Metab.1995; 39:42-51.
24. Lolodi O, Eriyamremu GE. Effect of Methanolic Extract of *Vernonia amygdalina* (Common Bitter Leaf) on Lipid Peroxidation and Antioxidant Enzymes in Rats Exposed to Cycasin. Pak J Biol Sci. 2013;16: 642-646.
25. Al-Otaibi SN, Alshammari GM, AlMohanna FH, Al-Khalifa AS, Yahya MA. Antihyperlipidemic and hepatic antioxidant effects of Leek leaf methanol extract in high fat diet-fed rats. All Life. 2020; 3(1):373-385.
26. Gaschler MM and Stockwell BR. Lipid peroxidation in cell death. Biochem Biophys Res Commun. 2017; 482:419-425.
27. Bezerra D, Militão G, de Morais M, de Sousa, D. The Dual Antioxidant/Prooxidant Effect of Eugenol and Its Action in Cancer Development and Treatment. Nutrients. 2017; 9(12):1367.
28. Ogbe RJ, Agbese SP, Abu AH. Protective effect of aqueous extract of *Lophira lanceolata* leaf against cisplatin-induced hepatorenal injuries and dyslipidemia in Wistar rats. Clin. Phytosci. 2020; 6(4):1-11.
29. Venkatachalam K, Vinayagam R, Arokia Vijaya Anand, M, Isa NM, Ponnaiyan R. Biochemical and molecular aspects of 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis: a review. Toxicol Res.2020; 9(1):2-18.