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Research Article

Effects of Alkaloids of *Cocos nucifera* Husk Fibre on Cardiovasular Disease Indices in Albino Mice

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ABSTRACT

Background: The fight against cardiovascular disease remains a challenge as it is a disease which affects the heart and blood vessels. The use of Cocos nucifera husk fibre in disease treatment in Nigeria is generally high. It is important to know the effect of the alkaloids of the plant in relation to cardiovascular diseases.

Methods: 48 albino mice with average weight of 18.28 ± 0.57 g were randomly divided into six groups (A to F) of eight albino mice each with group A as control while groups B,C,D,E,F and were administered 31.25, 62.5, 125, 250 and 500 mg/kg weight of the extract respectively. At the end of 7 days of administration, the animals were sacrificed and the serum was collected and various lipid parameters determined.

Results and Conclusion: The results revealed that the alkaloids caused a significant increase ($p<0.05$) in Triglyceride and Very low density lipoprotein concentrations at the doses of 62.5 and 125 mg/kg body weight compared to control. The alkaloids did not significantly alter ($p>0.05$) Serum total cholesterol and Low density lipoprotein-cholesterol concentrations but significantly reduced ($p<0.05$) High density lipoprotein–cholesterol concentration at all doses administered compared to control. The alkaloids caused a significant increase ($p<0.05$) in atherogenic index at the doses of 62.5 and 125 mg/kg the result suggest that the alkaloids of Cocos nucifera husk fibre may predispose subjects to cardiovascular diseases.

Keywords: Cardiovascular disease; *Cocos nucifera*; Alkaloids; Medicinal plants; Cholesterol; Lipoproteins

INTRODUCTION

Cardiovascular disease (CVD) refers to diseases of the heart and blood vessels, and include conditions such as coronary heart diseases (also know as isheamic heart disease), cerebrovascular disease (stroke {ischemic and hermorrhagic}), heart failure, rheumatic heart disease and hypertension (high blood pressure) [1]. The causes of cardiovascular disease are diverse but atherosclerosis and or hypertension are the most common [2]. Evidence suggests a number of risk factors for heart disease: age, gender, high blood pressure, tobacco smoking, excessive alcohol consumption, sugar consumption, family history, obesity, lack of physical activities, high serum cholesterol levels etc. [3].

Globally, cardiovascular diseases are the number one cause of death and they are projected to remain so, from an estimated 58million deaths in a 2005 survey, 17 million people died from cardiovascular disease, representing 30% of all global deaths however there is a difference in ranges from as high as 58% in Eastern Europe to as low as 10% in Sub Saharan Africa [4]. According to Gaziano et al. [5], the deaths rates in High Income countries are approximately 38% percent while in low and middle income countries, they have a lower CVD death rate of 28% and this could be associated with the of the whites.

It is important to recognize that a substantial proportion of these deaths (46%) were of people under 70 years of age in the more productive period of life; in addition, 79% of the disease burden attributed to cardiovascular disease is in this age group [6].

The fight against cardiovascular disease remains a challenge as it is a disease which affects the heart and blood vessels [1]. A critical challenge is observed in coronary disease; a disease of the heart and coronary arteries caused by the buildup of fatty

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materials in the blood vessels which supply the heart with oxygen.

It is also a challenge because free radicals are formed by the normal metabolic body processes such as breathing, and also by environmental contaminants and inadequate amounts of antioxidant, causes these free radicals to damage cells throughout the body. Part of this cellular damage leads to one of the major known factors in the development of heart disease, oxidation of cholesterol which contributes to the buildup of fatty plaque on artery walls (atherosclerosis), which can eventually slow or block blood flow to the heart [1].

Medicinal plants

Medicinal plants are plants which can be used to prevent and cure diseases, infection, and even relieve pain. They are considered a rich resource of ingredients which can be used in drug development and synthesis, these plants play a critical role in the development of human cultures around the whole world. According to Rasool [7], medicinal plants have many characteristics when used as a treatment and they include:

- **Synergic medicine:** The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.
- **Support of official medicine:** In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.
- **Preventive medicine:** It has been proven that the component of the plants also characterizes by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment.

Cocos nucifera

The Coconut Palm (*Cocos nucifera*) is one of man's most useful plants. The heavy crown of long flowing fronds and gently curved trunks lend a tropical effect to any landscape setting in which they can grow. A beautiful street tree, Coconut Palm is also ideal as a background tree, framing tree, or as a striking freestanding specimen. Coconut Palms located along streets, walkways or near patios require pruning to remove the flowers or developing fruit so it doesn't fall and cause injury or property damage, Cocos nucifera L. (is a plant commonly found along the south western Nigeria) is found throughout the tropics. Most commonly, it is sought for the flavor and nutritional qualities of its water obtained from fruit [8].

It is interwoven into the lives of the local people. It is particularly important in the low islands of the Pacific where, in the absence of land-based natural resources, it provides almost all the necessities of life food, drink, oil, medicine, fiber, timber, thatch, mats, fuel, and domestic utensils. For this good reason, it has been called the tree of heaven 'and tree of life'. Today it remains an important economic and subsistence crop in many small Pacific island states [9].

Coconut is believed to have its origins in the Indo-Malayan region, from whence it spread throughout the tropics. Its natural habitat was the narrow sandy coast, but it is now found on soils ranging from pure sand to clays and from moderately acidic to alkaline. It is non-invasive, and people have been largely responsible for its spread, particularly inland from its natural habitat. It thrives under warm and humid conditions but will tolerate short periods of temperatures below 21°C (70°F). Its crown of feather-like fronds and bunches of large fruits carried a top long slender stems makes it easily recognizable [9]. The fruit is a fibrous drupe. It consists of, from the outside in, a thin hard skin (exocarp), a thicker layer of fibrous mesocarp (husk), the hard endocarp (shell), the white endosperm (kernel), and a large cavity filled with liquid (water). When immature, the exocarp is usually green, sometimes bronze. Wide variation in fruit shape and size exist within types and populations. Fruit shapes vary from elongated to almost spherical and weigh between 850 g and 3700 g when mature [9]. The medicinal values include Anti Leishmanicidal activity [10], Antimalarial Activity [11], Anti-atherosclerotic effect Antimicrobial effect [12], Anticancer effect [12], Vasorelaxant and antihypertensive effect [13], electrolyte function [14].

Alkaloids

Alkaloids are the largest group of secondary chemical constituents made largely of ammonia compounds comprising basically of nitrogen bases synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring, most containing oxygen, this group also includes some related compounds with neutral and even weakly acidic properties [15].

Alkaloids show great variety in their botanical and biochemical origin, in chemical structure and in pharmacological action. Consequently, many different systems of classification are possible. For practical purposes it is useful, therefore, to maintain the well-established classifications based on chemical structures [15]. There are two broad divisions, which include:

- Non-heterocyclic or atypical alkaloids, sometimes called 'protoalkaloids' or biological amines, characterized by absence of the heterocyclic ring but also derived from amino acids [16], examples include mescaline, adrenaline and ephedrine [17].
- Heterocyclic or typical alkaloids, which is sub divided into two groups namely [17].

True alkaloids (characterized by a heterocyclic ring with a nitrogen atom, and are derived from amino acids) examples are atropine, nicotine and morphine [17].

Pseudo alkaloids (characterized by a heterocyclic ring with a nitrogen atom, but are not derived from amino acids), they include terpene-like and steroid-like alkaloids as well as purine-like alkaloids such as caffeine, theobromine and theophylline (Steroidal alkaloids) [16].

Bioavalability of alkaloids

Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals, plant especially higher plants contain about 10 to 25% of alkaloids [15]. Also alkaloids are found in certain types of fungi, such as psilocybin in the

fungus of the genus Psilocybe, and in animals, such as bufotenin in the skin of some toads [18].

MATERIALS and METHODS

Chemicals

Absolute n-Hexane, absolute ethanol, all assay kit were a product of Randis.

Animals

Forty-eight adult Swiss albino Mice with an average weight of 18.28 ± 0.57 g were obtained from the Animal Breeding Unit of the Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria. The animals were housed in standard plastic cages and acclimatized for a period of two weeks. They were maintained under standard conditions and had access to feed and water ad libitum.

Plant materials

Husk fibres of *Cocos nucifera* (West African Tall variety) dried at room temperature under shade were obtained from Nigeria Institute for Oil Palm Research (NIFOR), Badagry, Lagos State, in January, 2013. It was botanically authenticated at the institute by Mr. Igbene Collins (a NIFOR staff).

Preparation of extract

The extract was prepared according to the method of Adebayo et al. [11]. The samples were shade dried at room temperature and pulverized into powder. 500 grams (500 g) of the powder was percolated in 2.1 L of n-hexane for 72 h in a tightly stoppered glass container. This was shaken at intervals. The resulting mixture was filtered with Whatmann filter paper (110 mm). The residue was air dried, to allow complete evaporation of the n-hexane from the sample. This residue was again soaked in 3 L of absolute ethanol for another 72 h. This was filtered using Whatmann filter paper and the filtrate was concentrated using rotary evaporator at 40°C. This generated the alkaloid semisolid which was subsequently used for the experiment.

Extraction of alkaloid from *Cocos nucifera* husk fibre

160ml of the semisolid extract was 22 ml of 1M HCl added, after which it was basified by the addition of 6.5 ml of 5M NaOH yielding a white precipitate alongside with the solidification of the extract. The basified solution together with 18 ml of 0.9% NaCl and 125 ml of chloroform were separated with a separating funnel thrice, at each interval producing an upper (aqueous) layer and a lower (organic) layer. A total of three organic layers containing the alkaloid was concentrated in a water bath at a temperature of 370C. The percentage yield was 0.335% alkaloid.

Animal grouping and administration of extract

The forty-eight mice were randomly assigned into six groups (A-F), of 8 Mice each. Daily administration of 200 µl of the alkaloids of ethanolic of Cocos nucifera husk fibre was done orally for seven days.

Group A (Control): Administered appropriate volume of distilled water solution.

Group B-F: Administered 31.25 mg/Kg, 62.5 mg/Kg, 125 mg/Kg, 250 mg/Kg and 500 mg/Kg body weight of extract fraction respectively.

The extract fraction was dissolved in warm distilled water solution to form a suspension before administration.

LIPID PROFILE

Determination of total cholesterol concentration

The level of cholesterol was determined using enzymatic saponification procedure as described by Allain et al. [19].

Principle

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrene in the presence of phenol and peroxidase.

Cholesterol ester + H_2O ⟶ Cholesterol + Fatty Acids

Cholesterol + O_2 ⟶ Cholesterol-3-one + H_2O_2

$2H_2O_2$ + Phenol + 4-Aminoantipyrene ⟶ Quinoneimine + $4H_2O$

Procedure

The Cholesterol level was determined using appropriately diluted samples, 10 µl of each sample and 10 µl of standard with 1000 µl of reagents which was Mixed, incubated for 5 minutes at 37^0C, Absorbance was read at 546 nm.

Calculation

$$Concentration\ of\ cholesterol\ in\ Sample = \frac{\Delta Asample \times Concentration\ of\ Standard}{\Delta Astandard}$$

Determination of triglyceride (TRIGS) concentration

The level of Triglyceride was determined calorimetrically using the method described by Tietz [20].

Principle

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from Hydrogen Peroxide, 4- aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

Triglycerides + H_2O ⟶ Glycerol + Fatty Acids

Glycerol + ATP ⟶ Glycerol-3-Phosphate + ADP

Glycerol-3-Phosphate + O_2 ⟶ Dihydroxyacetone phosphate + H_2O_2

$2H_2O_2$ + 4-Aminophenazone + 4 Chlorophenol ⟶ Quinoneimine + HCl + $4H_2O$

Procedure

The triglyceride level was determined using appropriately diluted samples, 10 µl of each sample and 10 µl of standard wit 1000 µl of reagents which was Mix, incubated for 5 minutes at 37°C, Absorbance was read at 546 nm.

Calculation

$$Concentration\ of\ TRIGS\ in\ Sample = \frac{\Delta Asample \times Concentration\ of\ Standard}{\Delta Astandard}$$

Determination of HDL-cholesterol concentration

The HDL level was determined using the method described by Bachorik.

Principle

Low density lipoprotein (LDL and VLDL) and Chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (High Density Lipoprotein) fraction, which remains in the supernatant, is determined.

Procedure

The HDL Cholesterol level was determined using appropriately diluted samples, 500 µl of sample and 1000 µl of precipitant pipetted into centrifuge tubes which was then mixed and allowed to sit for 10 minutes at room temperature Centrifuged at 4000rpm for 10 minutes. The clear supernatant was separated within 2 hours and the cholesterol content was determined using appropriately diluted samples, 100 µl of each sample and 10 µl of standard wit 1000 µl of reagents which was Mix, incubated for 5 minutes at 370C, Absorbance was read at 546 nm.

Calculation

$$Concentration\ of\ HDL\ cholesterol\ in\ Sample\ (mmol/l) = \frac{\Delta Asample \times Concentration\ of\ Standard}{\Delta Astandard}$$

Determination of LDL-cholesterol concentration

The LDL Cholesterol level was calculated using the formulae as described by Friedewald et al., The Friedewald equation is as follows:

LDL Cholesterol (mmol/l) = Tot. Chol-VLDL-HDL Chol

Determination of VLDL-cholesterol concentration

The value of VLDL Cholesterol is determined using the formulae described by Friedewald et al., (1972). The equation is as follows:

$$VLDL\ (mmol/l) = \frac{TRIGS}{2.2}$$

Determination of atherogenic index

The atherogenic index was determined using the following methods.

The Atherogenic Index as described by Lamarche, 1996

$$AI = \frac{TOT.CHOL}{HDL}$$

Statistical analysis

Experimental data are presented as Mean ± Standard error of mean (SEM). Statistical analysis was implemented using computer software SPSS 20.0 version statistical package program (SPSS, Chicago, IL). One-way analysis of variance was used to compare variables among the different groups. Level of significance (Post hoc comparisons) among the various treatments was determined by Duncan's Multiple Range Test. The values were considered statistically significant at $p<0.05$.

RESULTS

Cardiovascular diseases

Serum lipid profile:

Triglyceride concentration: The administration of alkaloids of Cocos nucifera husk fibre caused a significant increase in triglyceride concentration of 62.5 and 125 mg/kg body weight while other doses did not (Table 1).

Total cholesterol concentration: The administration of alkaloids of Cocos nucifera husk fibre caused significant change ($p>0.05$) in cholesterol concentration compared to control (Table 1).

Very low density lipoprotein concentration: The administration of alkaloids of Cocos nucifera husk fibre significantly increased ($p<0.05$) (Table 1) at the doses of 62.5 and 125 mg/kg body weight while other doses compared with control did not.

High density lipoprotein-cholesterol concentration: The administration of alkaloids of Cocos nucifera husk fibre causes a significant reduction ($p<0.05$) in the serum HDL concentration at all doses compared to control (Table 1).

Low density lipoprotein-cholesterol concentration: The administration of alkaloids of Cocos nucifera husk fibre at all doses caused no significant alteration ($p<0.05$) to the LDL-cholesterol concentration compared to control (Table 1).

Atherogenic Index

Table 1: Serum lipid profile in mice administered alkaloids of Cocos nucifera husk fibre.

GROUP Mg/kg	TRIGS (mmol/l)	CHOL (mmol/l)	VLDL (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	AI (mmol/l)

Control	14.20 ± 1.30^{ab}	371.40 ± 10.10^{a}	6.45 ± 0.61^{ab}	140.84 ± 6.0^{d}	224.00 ± 13.3^{ab}	3.40 ± 0.51^{a}
31.25	17.00 ± 2.20^{b}	336.00 ± 45.80^{a}	7.70 ± 1.00^{ab}	110.80 ± 4.40b^{c}	217.52 ± 49.10^{ab}	3.39 ± 0.65^{a}
62.5	31.15 ± 7.70^{d}	304.15 ± 29.46^{a}	12.75 ± 3.48^{c}	116.40 ± 5.60 ^{c}	200.00 ± 12.00^{a}	6.34 ± 0.04^{b}
125	22.62 ± 1.50^{c}	357.60 ± 74.40^{a}	5.20 ± 0.70^{ab}	92.40 ± 1.57^{a}	281.61 ± 10.00^{b}	2.94 ± 0.21^{a}
250	9.70 ± 1.33^{a}	379.200 ± 10.40^{a}	5.20 ± 0.70^{ab}	92.40 ± 1.57^{a}	281.61 ± 10.00^{b}	3.06 ± 0.51^{a}
500	9.01 ± 2.00^{a}	393.30 ± 18.20^{a}	3.10 ± 1.00^{a}	102.67 ± 3.83^{ab}	287.54 ± 21.00^{b}	3.29 ± 0.52^{a}

TRIGS: Triglyceride; CHOL: Cholesterol; VLDL: Very Low density lipoprotein; HDL: High Density lipoprotein; LDL: Low Density Lipoprotein

Values are expressed as mean ± SEM (n=8). Values in the same column are not significantly different at $p<0.05$ whereas values with different alphabets are significantly different.

DISCUSSION

Alterations in the concentration of the major lipids like Total Cholesterol, triglycerides, high density lipoprotein, low density lipoprotein and very low density lipoprotein can give useful information on the lipid metabolism as well as predisposition of the subject to atherosclerosis and its associated coronary heart diseases [21].

High serum cholesterol concentrations are an important risk factor for cardiovascular diseases. An increase of 1% serum cholesterol is reported to have resulted in a 3% increase in coronary heart disease [22]. At doses of extracts of alkaloid administered, there was no significant change in total cholesterol indicating that the alkaloids of Cocos nucifera husk fibre may not adversely affect cholesterol metabolism.

Low density lipoprotein Cholesterol is often designated a bad cholesterol since high levels of it in the serum are linked with increased deposition of cholesterol in the arterial walls. Low density lipoprotein transport cholesterol from its site of synthesis in the liver to various tissues and body cells where it is separated and used by the cells. A reduction in LDL-cholesterol by 2 mg/dl or 0.1 mmol/l can result in 1% reduction in the risk for coronary artery disease. The results suggest the alkaloids caused no significant alteration to serum LDL-cholesterol, it also suggest that the alkaloids may not adversely affect the normal distribution of cholesterol to peripheral tissues.

High Density Lipoprotein Cholesterol (HDL-C) mediates the removal of cellular cholesterol and its secretion into the bile by the liver [23-25]. The alkaloids reduced serum with high HDL-C, suggesting that the basic role of HDL in transporting cholesterol from peripheral tissues to the liver for metabolism and excretion may be adversely affected.

The results also revealed that the alkaloids may increase VLDL and TAG at the doses of 62.5 and 125 mg/kg body weight suggesting that they may enhance the synthesis of TAG in the liver at these doses.

CONCLUSION

The study evaluated the effect of alkaloids of Cocos nucifera husk fibre extract on cardiovascular disease indices.

The results of the study suggest the following

- The alkaloids of Cocos nucifera husk fibre may increases the triglyceride concentration in the serum at moderate doses.
- The alkaloids of Cocos nucifera husk fibre increases the synthesis concentration of very Low Density Lipoprotein in the liver at moderate doses.
- The alkaloids of cocos nucifera may not adversely affect cholesterol synthesis and its distribution to peripheral tissues but may adversely affect its retrieval from peripheral tissues to the liver by HDL.
- The alkaloids of *cocos nucifera* husk fibre may adversely affect cholesterol homeostasis and predispose to cardiovascular disease at the long run.

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