ANTIOXIDANT PROPERTY AND CARDIOVASCULAR EFFECTS OF COCONUT (*Cocos nucifera*) WATER.

ABSTRACT

Coconut water, a natural nutritious beverage that contains several biologically active chemical substances, is used in the management/treatment of several disorders in Eastern Nigeria. This study was set up to evaluate the antioxidant potential of coconut water and its effect on the cardiovascular system in albino rats. Twenty five adult male albino rats, used in this work, were placed into five groups (A, B, C, D and E), of five rats per group. Groups A, B, C and D were administered orally with 0.5, 1.0, 1.5 and 2.0ml/kg body weight respectively of the coconut water for fourteen consecutive days. Group E was the control. Glutathione peroxidase (GPX), superoxide dismutase (SOD) and Malondialdehyde (MDA) levels were used to investigate antioxidant activity of the coconut water, while lipid profile was determined as an index of its cardiovascular effect. GPX and SOD activity was significantly higher ($P < 0.05$) in the test groups than in untreated group, while MDA levels decreased significantly ($P< 0.05$) in the treated groups relative to the control. There was a significant reduction ($P< 0.05$) in total cholesterol, triglycerides and low density lipoprotein, while high density lipoproteins increased significantly ($P< 0.05$) in the test animals relative to the control. These observations were found to be linearly dose-dependent. These results may be due to the chemical constituents of the coconut water, and could be partly responsible for its application in the management of some disorders.

Keywords: Antioxidant enzymes, total cholesterol, lipoproteins, serum, albino rats.

INTRODUCTION

The coconut palm (*Cocos nucifera*), found throughout the tropics and subtropics, is one of the most widely distributed edible fruit- bearing palms in the world. It is a large palm, growing up to 30 meter (98ft) tall, with pinnate leaves 4-6 meters (13-20) long and pinnae 60-90cm long. Coconuts are generally classified in to two type: tall and dwarf (Pradeepkumar, 2008). Coconut water is a natural liquid that contains many biologically active compounds. These include numerous antioxidant compounds that have the ability to scavenge free radicals in the body. It also contains cytokins, a plant chemical which has shown anti-aging and anti-carcinogenic effects. Coconut water also contains B vitamins, which are water soluble and are required for cellular functions (Depeint *et al.*, 2006). Coconut water contains a variety of inorganic ions such as calcium, magnesium, phosphorus, sodium, potassium and selenium (Charles, 2012). Further, other components found in coconut water include sugars, sugar alcohols, lipids, amino acids, nitrogenous compounds, organic acids and some enzymes. They play different functional roles in plant and human systems due their distinct chemical properties (Kathleen and Zelman, 2011).

Many studies have shown that the antiviral, antibacterial, anti-inflammatory and antioxidant activities of coconut water may help ease a number of minor to severe health conditions. This nutrient rich drink has been used to regulate blood pressure, blood sugar, and cholesterol levels, and it has been found to boost energy levels and increase metabolism in human body. Other conditions that it has been found to be effective in treating include stomach flu, dysentery, indigestion, constipation, intestinal worms, urethra stones, malfunctioning kidneys, dry and itchy skins, age spot and wrinkles (Young and Ge, 2009). Similarly, some recent studies have found that coconut water can help increase high density lipoprotein (good) cholesterol, which makes it a wonderful natural treatment for maintaining good cardiovascular health. Young coconut water has estrogen-like characteristics. It mixes easily with blood, and was used during World war II in emergency transfusions (Anurage and Rajamohan, 2003).

Coconut water can also serve as emergency short-term intravenous hydration fluid. This is possible because it contains a high level of sugar and other salts that make it possible to be used in the bloodstream, much like the modern lactate Ringer Solution or a dextrose/water solution as an intravenous solution (Kalman and Feldman, 2012). In Eastern Nigeria, coconut water is used for several medicinal purposes which include management and treatment of various disorders such as gastrointestinal disorders, high blood pressure, dehydration, kidney malfunction, anxiety, etc.

Glutathione peroxidase (GPX) is an enzyme which acts on lipid hydroperoxide (LHP) substrates that are released from membrane phospholipids by phospholipaseA_2. It can utilize cholesterol hydroperoxide and hydrolyzes hydrogen peroxide (H_2O_2) at low concentration (Finn *et al.*, 2009). The antioxidant enzyme, GPX, catalyze the reaction of H_2O_2 and hydroperoxides formed from fatty acid, thereby effectively removing toxic peroxides from living cells. It plays the important role of protecting cells from potential damage by free radicals, formed by peroxide decomposition (Khan *et al.*, 2010).

Lipid peroxidation is an established mechanism of cellular injury, and is used as an indicator of oxidative stress. Polyunsaturated fatty acids peroxides generate malondialdehyde (MDA) and 4-hydroxyalkanals upon decomposition (Esterbauer *et al*., 1991). Superoxide dismutase (SOD) decomposes superoxide anion into hydrogen peroxide and oxygen at very high rates. Superoxide radical is involved in diverse physiological and patho-physiological processes (Johnson and Giulivi, 2005). Lipid profile is a general term that is given to tests for high density lipoprotein,

low density lipoprotein, total cholesterol and triglycerides. A shift in the normal level of any of these components of lipid profile is of interest to cases of cardiovascular disorders (Burtis et al., 2008).

Documented scientific evidence on the various medicinal applications of coconut water has been scarce in this part of the world. Hence, the present study investigated the antioxidant property of coconut water and its possible effect on the functionality of the cardiovascular system.

MATERIALS AND METHODS

The chemicals used in this research were of analytical grade.

Preparation of coconut water:

Coconuts fruits were plucked from a tall matured coconut tree at Amuda in Ezza North Local government area of Ebonyi State. They were identified by Prof. S. C. Onyekwelu of Applied Biology Department, Ebonyi State University, Abakaliki, Nigeria. Each coconut seed was punctured at the holes using a sterilized nail. It was then placed over a container and allowed the water to drain. The coconut water was obtained fresh and used immediately.

Animals and handling:

Ethical approval was given by Ebonyi State University Research and Ethics Committee.

Twenty-five adult male albino rats, weighing 140-155g were bought from the animal house of Biochemistry Department, University of Nigeria, Nsukka. They were placed in five groups (A-E) of five rats in each group, and kept in animal house of Biochemistry Department, Ebonyi State University Abakaliki for seven days to acclimatize. All the rats were allowed free access to feed (rat chaw) and water before and throughout the experiment. Groups A, B, C and D were treated orally with 0.5, 1.0, 1.5 and 2.0 ml/kg body weight respectively of the coconut water for fourteen consecutive days. Group E was the control. Blood samples were collected from the animals following an overnight fast through cardiac puncture under mild anaesthesia using diethylether.

Measurement of antioxidant property:

The method of Ohkawa et al (1979) was used to measure the level of MDA. SOD and GPX activities were determined by the methods of Kakkar and Viswanathan (1984) and Tappel (1978) respectively.

Assessment of cardiovascular effect:

The enzymatic method of determination of cholesterol concentration described by Burtis *et al.*, (2008) was adopted for measurement of total cholesterol,while the methods described by ochei and kalhalkar (2007) were used to determine the levels of HDL and triglycerides. LDL concentration was calculated from Friedewald equation as stated by Burtis *et al.*, (2008).

Data Analysis:

Statistical analysis was done using analysis of variance (ANOVA). Means were compared for significance using Duncan's multiple range test ($P<0.05$)(Sokal and Rholf, 1969).

RESULTS AND DISCUSSION

There was a noticeable increase in physical activities and rate of food and water intake in the groups given the coconut water compared with the control (data not shown). We are currently investigating the possible mechanisms of these observations. Chemical constituents reported in coconut water have been shown to influence these processes. For example, recent studies have shown coconut water is equally as effective carbohydrate-electrolytes sports drink in replenishing fluids after exhaustive exercise and it is also superior to water and sports drink options (Kalman and Feldman, 2012). Inorganic ions are required for normal cellular functions, and are critical for enzyme activation, bone formation, hemoglobin function, gene expression, and the metabolism of amino acids, lipids and carbohydrates (Wall, 2006). The recorded significant increase ($p<0.05$) in the activity of glutathione peroxidase and superoxide dismutase in the test groups relative to the untreated group (Table 1) may be attributed to the antioxidant constituents of the coconut water. Coconut water contains a wealth of micronutrients such as inorganic ions and vitamins that enhance the natural antioxidant system of the body. These micronutrients acts directly in the body to quench free radicals that can damage cells or they can indirectly increase the production of antioxidant enzymes (such as superoxide dismutase, catalase and glutathione peroxidase) that promote the removal of damaging radicals (Kalman and Feldman, 2012).

The levels of lipid peroxidation marker, malondialdehyde (MDA) in the treated groups were significantly lower ($p<0.05$) than those in the control group. This may be due to the corresponding increase in the activity of glutathione peroxidase and other antioxidant body systems. This result is consistent with the report of Evans and Halliwel (2001); Shenken (2006), that hyper-metabolism gives rise to an increased production of reactive oxygen species (ROS) or

free radicals as a result of increased oxidative damage to the various components of human cell, especially the polyunsaturated fatty acids in the nucleus. Fortunately, living organisms have well developed antioxidant systems to neutralize the most detrimental effects of these oxidizing free radicals by donating electrons or indirectly as a part of metalloenzymes (a diverse class of enzymes that require a catalytic metal ion for their biological activity) such as glutathione peroxidase (selenium-present in coconut water) or superoxide dismutase (zinc, copper) to catalyse the removal of oxidizing species (Shenken,2006).

There was a significant reduction ($p<0.05$) in total cholesterol, triglycerides and LDL, and significant increase ($p<0.05$) in HDL in the treated groups relative to the control (table 2). This confers cardiovascular protective property to the coconut water. The chief role of these lipid compounds (except HDL) in pathological processes is as factors in the genesis of atherosclerosis of vital arteries, causing cerebrovascular, coronary and peripheral vascular disease (Murray et al., 2003). Epidemiological studies have shown that high levels of HDL have protective value against cardiovascular diseases such as ischemic stroke and myocardial infarction (Philip, 2007). The exact biochemical mechanism involved in the reduction of total cholesterol, triglycerides and LDL, and increase in HDL by the coconut water is presently not well understood. According to Agbafor and Akubugwo (2007), the hypocholesterolaemic effect of ethanolic extract of *Cymbopogon citratus* may be ascribed to modification of cholesterol uptake from the intestine, conversion of cholesterol to bile acids and increased excretion of bile acids. Anurage and Rajamohan (2003) showed that coconut water has cardioprotective effect in experimental myocardial infarction induced in rats and this was probably attributed to the rich of mineral ions in coconut water ,especially potassium.

Based on the results of this research, consumption of coconut water may boost body's antioxidant systems, which neutralize the effects of free radicals, and reduce the risk of cardiovascular disorders. These results, therefore, not only make coconut water popular to consume as refreshing beverage but also valuable in health and medicine. We are presently investigating the possible biochemical mechanisms responsible for our major findings.

Table 1: Levels of (GPX), (MDA) and SOD in the albino rats after fourteen days of treatment.

Group	GPX Activity(U/l)	SOD,activity(U/mg protein)	MDA conc. (nmol/ml)
A	324.80±2.10^{d}	8.05±0.70^{e}	5.75±0.33^{c}
B	383.86±2.74^{d}	15.30±1.15^{d}	4.47±0.41^{c}
C	408.81±3.52^{c}	27.62±1.42^{c}	3.81±0.15^{d}
D	495.27±3.14^{b}	36.09±2.29^{b}	2.21±0.11^{b}
E	180.43±3.34^{a}	4.20±0.21^{a}	9.11±0.81^{a}

All values are Mean ± Standard deviation; n=5 Values in the same column having different superscripts are significantly different (P < 0.05).

Table 2: Concentrations of total cholesterol, triglycerides, high density lipoprotein and low density lipoprotein of the animals after fourteen days of treatment.

Animal group	Total cholesterol (mg/dl)	Triacylglycerol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
A	224.87± 1.41^{d}	128.38±2.2^{b}	20.80± 3.1^{a}	178.39±2.14^{b}
B	193.87±3.02^{d}	121.51± 1.7^{b}	24.70±2.1^{a}	174.87± 1.35^{b}
C	121.81±1. 90^{c}	116.69± 4.0^{b}	32.24±2.2^{b}	124.83±1.29^{c}
D	81.60±2.44^{b}	91.38± 6.2^{c}	38.36± 2.4^{b}	50.11±2.15^{d}
E	263.32±2.10^{a}	174.87±3.06^{a}	18.46± 2.1^{a}	189.88± 5.9^{a}

Values are mean ± standard deviation, n=5. Values in same column with different superscripts differ significantly (P<0.05).

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