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Research Article

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Effect of Coconut Water on Lipid Peroxidation and Some Antioxidant Status of Diabetic Rats

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Abstract

Aim: *The antioxidant efficacy of tender and mature coconut water of Cocos nucifera fruit was studied to ascertain the potential effects of the liquid on some biochemical indices.*

Materials and Methods: *In vitro antioxidants activity was carried out on the coconut water using DPPH assay, ferric ion reducing antioxidant power assay (FRAP), and phosphomolybdenum assay (PM). Twenty albino rats (80 – 120 g) divided into 5 groups of 4 rats each were used for this study. Group 1 served as the normal control while groups 2-5 were induced with diabetes using 120 mg/kg b.w. alloxan monohydrate. Group 2 was untreated while Group 3 was treated with glibenclamide. Groups 4 and 5 were treated with 1 ml/kg b.w of mature coconut water and tender coconut water respectively.*

Results: *Both tender and mature coconut water and that of the standard (ascorbic acid) showed a concentration-dependent DPPH scavenging capacity. The liquid also reduced the ferric ion in a concentration-dependent manner. Both tender and matured coconut water reduced Phosphate-Mo (VI) to Phosphate Mo (V) in a concentration-dependent manner. Malondialdehyde concentration (MDA) decreased significantly ($p<0.05$) when compared to group 2. The in vivo antioxidant status (CAT, GSH, Vitamin C, and Vitamin E) showed a significant ($p<0.05$) increase unlike group 2 while SOD activity showed significant ($p < 0.05$) increase in group 2.*

Conclusion: *The results obtained from this study showed that both tender and mature coconut water have potent antioxidant properties and as such can reduce the complications associated with diabetes mellitus.*

Keywords: *lipid peroxidation, diabetic rats, Cocos nucifera, alloxan, antioxidant, In vitro*

Introduction

In today's world, diabetes mellitus is still a major deteriorating heterogeneous metabolic disorder [1]. According to World Health Organization, WHO, diabetes has affected approximately 422 million people worldwide [2]. It is projected that death due to this will be double between 2005 to 2030 This noncommunicable illness has grown in prominence in Sub-Saharan Africa, with Nigeria being the most affected, with approximately 4 million diabetics [3, 4]. In patients with Diabetes mellitus, its complications are the major cause of morbidity and mortality [5]. Diabetes mellitus is a metabolic condition marked by a loss of glucose homeostasis caused by abnormalities in insulin production, insulin action, or both, resulting in impaired glucose metabolism and other energy-producing fuels like lipids and protein [6, 7].

Several pathogenic processes are involved in the development of diabetes ranging from autoimmune destruction of the pancreatic B-cells with consequent insulin deficiency (Type 1 diabetes) to abnormalities that result in resistance to insulin action (Type 2 diabetes) [8].

High levels of free radicals combined with a reduction in antioxidant defences can cause damage to cellular organelles and enzymes, increased lipid peroxidation, and the development of diabetes complications, all of which have been linked to oxidative stress [9].

Coconut water (CW) is a natural nutritious beverage which can be considered as a functional food/nutraceutical as it contains several biologically active components and possess cardioprotective, hepatoprotective, hypolipidemic and antihypertensive properties in experimental animals [6]. This juice is mostly consumed locally as fresh in tropical areas since it deteriorates easily once exposed to air [10].

Antioxidant activities of polyphenolics derived from CW have claimed beneficial health functions for hypoglycaemic effect, retarding aging and preventing cancer and cardiovascular diseases [11].

This research was aimed at investigating the effect of coconut water on lipid peroxidation and some antioxidant status on alloxan-induced diabetic rats.

Methods

Plant Material: *The Cocos nucifera* fruits used for this study were collected from Mr Nwaeze's compound of Echara Nsukka, in Nsukka local government area. The fruits were identified and authenticated by Mr. A. Ozioko of the Bioresource Department and Conservation Program (BDCP), Research Centre, Nsukka.

Preparation of Plant Materials: The fresh tender coconut of Cocos nucifera fruit between 5-6months and mature coconut of Cocos nucifera fruit between 10-11 months were harvested from the same coconut plant all through the project work. The husk of the fresh coconut was removed and the shell was cut open to collect the coconut water.

Animals

Adult male rats Wistar strain (80 – 120 g) and adult Swiss albino mice (20 – 30 g) of both sexes obtained from the animal holding unit of the Department of Zoology and Environment Biology, University of Nigeria, Nsukka were used in the study. The animals were housed under standard conditions (25 ± 2 0C and 12 hours light/dark cycle). The rats and mice were fed two times in a day with standard pellets (Grand Cereals Ltd, Enugu Nigeria) and had unrestricted access to clean drinking water.

Qualitative Phytochemical Analysis of Tender and Mature Coconut Water of *Cocos Nucifera*

The qualitative phytochemical analysis was done using the methods of Trease and Evans (1989).

Quantitative phytochemical Analysis of Tender and Mature Water of *Cocos Nucifera* Fruit

The quantitative phytochemical analysis was done using the method of Harborne (1973).

Antioxidant Activity by DPPH Assay

The radical scavenging activity was determined by the spectrophotometric method based on the reduction of MeOH solution of 2,2-diphenyl-1- picrylhydrazyl (DPPH) [11].

Ferric ion reducing antioxidant power assay (FRAP)

Ferric ions reducing power was measured according to the method of [12].

Total Antioxidant capacity by Phosphomolybdenum Assay (TAC)

Total antioxidant activity was estimated by phosphomolybdenum assay according to [12].

Acute Toxicity Studies (LD_{50})

The acute toxicity studies of the fresh tender and mature coconut water were estimated in mice using the method of [13].

Experimental Design: The study was carried out in stages as follows:

Animal Grouping

Twenty albino rats divided into five groups of four rats each were used for this study.
Group 1: Control (non-diabetic, received normal feed orally).
Group 2: Positive control (diabetic infected rats and untreated)
Group 3: Standard control (diabetic infected rats treated daily with 0.6 mg/kg body weight glibenclamide)
Group 4: Diabetic infected rats treated daily with 1ml/kg body weight mature coconut water
Group 5: Diabetic infected rats treated daily with 1ml/kg body weight Tender coconut water

Measurements of biochemical parameters

The glucose levels in the rat serum were measured by the glucose oxidase method using a standard reagent kit. Lipid peroxidation was performed measuring the level of the lipid peroxidation product, malondialdehyde (MDA) using spectrophotometer as described by [14].

Superoxide dismutase activity was assayed by the method described by [15]. The activity of catalase was assayed by the method of [16]. Glutathione concentration was determined according to the method of [17]. Vitamin C was determined according to the method of [18].

Vitamin E was determined according to the method [19].

Results

Quantitative and Quantitative Phytochemical Composition of Tender and Mature Coconut Water of *Cocos nucifera* Fruit

The preliminary qualitative and quantitative analysis of tender coconut water of Cocos nucifera revealed the presence of Glycoside, saponin and acidic compounds in relative amount while phenol, terpenoid, steroid were present at moderate level. Reducing sugar, alkaloid and flavonoids, were present in high quantity. Tannins were not detected. The mature coconut water revealed relative amount of Glycoside, saponin, reducing sugar and acidic compounds while tannins, phenol and terpenoids, were present at moderate level. Alkaloids, flavonoids and steroid were present in

high quantity. Quantitatively, the tender and mature coconut water constitutes respectively; alkaloids (212.42 ± 125.59 and 385.08 ± 33.50 mg/ml), flavonoids (683.8 ± 62.49 and 490.2 ± 43.65 mg/ml), terpenoids (91.32 ± 19.55 and 123.33 ± 45.13 mg/ml) and glycoside (14.95 ± 0.82 and 7.33 ± 0.48 mg/ml) and total phenolics (134.70 ± 11.0 and 67.2 ± 10.0 mg/ml). Tannins (22.72 ± 1.054 mg/ml) in mature coconut water but was not detected in tender coconut water (Table 1).

Radical-quenching abilities of the Tender Coconut water, Matured Coconut water of Cocos nucifera and ascorbic acid at different concentrations were tested using DPPH radicals. The liquid showed a significant concentration-dependent DPPH scavenging capacity with a significant ($p < 0.05$) difference from that of the ascorbic acid standard. The observed potent DPPH radical-scavenging capacity of the liquid (tender and mature coconut water) corroborated with the various concentrations of the standard (ascorbic acid) with a good positive correlation coefficient (R2) of 0.531, 0.108 and 0.901, and IC50 of 1.87 mg/ml, 2.49 mg/ml and 0.27mg/ml respectively (Figure 1).

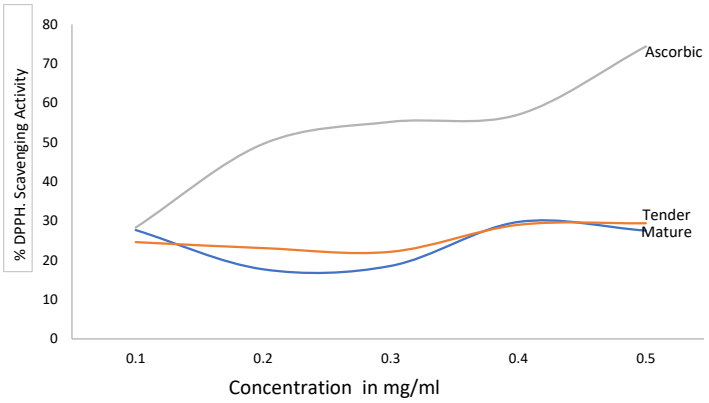

Figure 1: Free radical-scavenging activity of Matured and Tender coconut water of and ascorbic acid on 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH.)

Table 1: Results of the Qualitative and Quantitative Phytochemical Composition of tender and mature coconut water of Cocos nucifera

Phytochemical Constituents	Qualitative and Quantitative Phytochemical Composition of Tender and mature coconut water of *Cocos nucifera*			
	Tender	Mature	Tender (mg/ml)	Mature (mg/ml)
Alkaloids	+++	+++	212.42±125.59	385.08 ± 33.50
Flavonoids	+++	+++	683.8 ± 62.49	490.2 ± 43.65
Terpenoids	++	++	91.32 ± 19.55	123.33 ± 45.13
Glycoside	+	+	14.95 ± 0.82	7.33 ± 0.48
Phenolics	++	+	134.70 ± 11.00	67.2 ± 10.0
Tannin	ND	++	ND	66.0 ± 3.0
Saponin	ND	++		
Steroids	+++	+		
Reducing sugar	+++	+		
Acidic compds.	+	+		

Key

+ slightly present
++ moderately present
+++ highly present
N.D Not detected

The liquid (tender and mature coconut water) was able to reduce ferric ion in a concentration-dependent mode with no significant ($p > 0.05$) difference from those of the ascorbic acid standard. There is also no significant ($p > 0.05$) difference between the reducing capacities of the liquid and those of the standard (ascorbic acid) at the various concentrations. The ferric ion reducing activity of the coconut water correlated with the concentrations of the standard (ascorbic acid) with a good positive correlation coefficient (R^2) of 0.93, 0.693 and 0.311, and (IC50 = 92.56, 137.39 and 233.79 mg/ml) respectively (Figure 2).

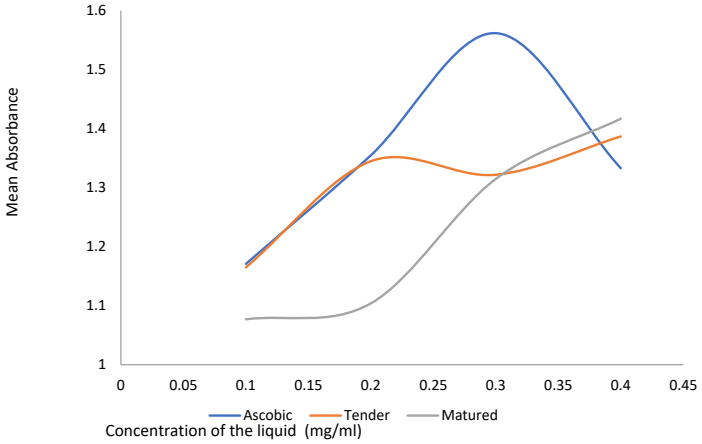

Figure 2: Ferric ion reducing antioxidant power assay (FRAP) of tender, matured coconut water of *Cocos nucifera* and ascorbic acid

Figure 3 shows that the liquid (tender and mature coconut water) was able to reduce Phosphate-Mo (VI) to Phosphate Mo (V) in a concentration-dependent mode with no significant ($p > 0.05$) difference from those of the standard (ascorbic acid). There is also no significant ($p > 0.05$) difference between the reducing capacities of the liquid and those of the standard at various concentrations. The phosphomolybdenum reducing activity of the liquid has a good positive correlation coefficient with a good positive correlation coefficient (R2) of 0.986, 0.799 and 0.887 and (IC50 = 39.56, 48.39 and 19.89 mg/ml) respectively the liquid was found to be powerful in reduction of Phosphate Mo (VI) to Phosphate Mo (V).

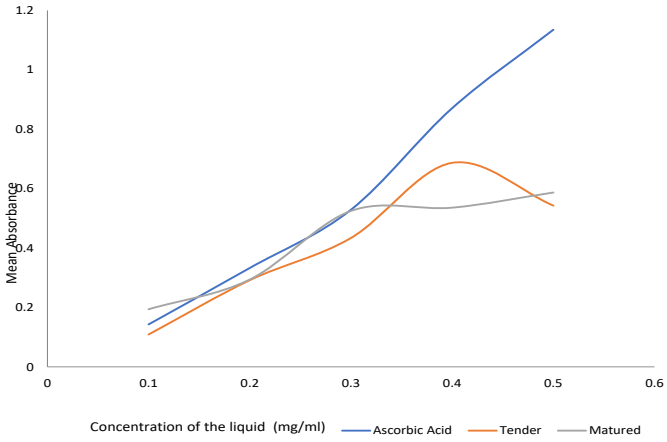

Figure 3: Total antioxidant activity of Tender, Matured Coconut water and Ascorbic acid estimated by phosphomolybdenum assay

The acute toxicity test of the tender and mature Coconut Water of Cocos nucifera fruit showed no death or adverse reaction up to 5ml/kg body weight. This showed the relative safety or non-toxic nature of the liquid at that concentration range (Table 2).

Table 2: Phase I and II of the acute toxicity testing of Tender and Mature *Coconut Water* of Cocos nucifera fruit

Phase/Groups	Dosage of extract (ml/kg b.w)	Mortality rate
Phase 1		
Group 1	0.01	0/3
Group 2	0.10	0/3
Group 3	1.00	0/3
Phase II		
Group 1	1.60	0/3
Group 2	2.90	0/3
Group 3	5.00	0/3
n = 3		

Table 3 shows the glucose concentration of rats treated with tender and mature coconut water of Cocos nucifera fruit. The results revealed that there was non-significant ($p>0.05$) difference among the test groups (4 and 5). However, glucose concentrations of rats in group 3, 4 and 5 were found to be significantly ($p<0.05$) lower than that of group 2 rats that were infected but not treated.

Table 3: Effect of Tender and Mature Coconut Water of Cocos nucifera Fruit on the glucose concentration of Alloxan Induced diabetic Rats

Treatment Group	Glucose Conc. Before Induction (mg/dl)	Glucose Conc. After Induction (mg/dl)	Glucose Conc. After Treatment (mg/dl)
Group 1	50.25 + 5.18aa	50.25 +5.18aa	71.75 +10.21ab
Group 2	61.25 + 16.11aa	488.00 +115.13cb	161.00 +56.76bb
Group 3	55.50 + 11.32aa	469.25 +183.97cb	83.25 +13.30aa
Group 4	54.00 + 12.93aa	272.25 +55.53bb	86.50 +21.61aa
Group 5	52.50 + 3.10aa	324.00 +121.80bb	81.00 +11.69aa

Malondialdehyde (MDA) concentration was found to be non- significantly ($p>0.05$) difference when compare among the test group (group 4 and 5). However, group 4 and 5 were found to be significantly ($p<0.05$) lower than group 2 (untreated rats). The effects of the liquid (tender and mature coconut water) on SOD revealed that there was significant ($p<0.05$) increase when group 4, and 5 were compared to group 2 (untreated mice). However, group 3 also was found to be significantly ($p<0.05$) higher than group 2 (untreated rats). Catalase activity was observed to be insignificant ($p>0.05$) when group 3, 4 and 5 were compared to themselves. However, there was a significant ($p<0.05$) increase in groups 4 and 5 compared to group 2 (untreated rats). The effects of the liquid on GSH revealed that Groups 4 and 5 were found to be significantly ($p<0.05$) higher than group 2. Vitamin C concentration was found to be significantly ($p<0.05$) higher when group 4 and 5 was compared to groups 1 and 3 rats, the normal and standard control groups respectively. However, group 4 and 5 were found to be significantly ($p<0.05$) higher than group 2 (untreated rats). The effects of the liquid on Vitamin E revealed that there was significant ($p<0.05$) difference when group 4, and 5 were compared to group 2. However, group 3 also was found to be significantly ($p<0.05$) higher than group 2 (Table 4).

Discussion

The preliminary qualitative and quantitative analyses of tender coconut water of Cocos nucifera revealed the presence of glycoside, saponin and acidic compounds in relative amount while phenol, terpenoid, steroid were present at moderate level. Reducing sugar, alkaloid and flavonoids, were present in high quantity. Tannins were not detected. The mature coconut water revealed relative amount of glycoside, saponin, reducing sugar and acidic compounds while tannins, phenol and terpenoids, were present at moderate level. Alkaloids, flavonoids and steroid were present in high quantity. This is consistent with the result of except for tannins that was detected only in mature coconut water [20]. According to the findings, mature coconut water has more alkaloids, terpenoids, and tannins than tender coconut water, whereas tender coconut water includes more phenol, flavonoid, and glycosides. When taken by animals, the presence of these biologically active compounds suggests that the plant could serve as a potential source of drugs, and their secondary metabolites could have biological activities such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation, modulation of hormone metabolism, and anticancer property [21].

Alkaloids have been shown to have many biological activities including antioxidant activity, hypoglycemic effect in diabetic patients, antihypertensive effects and anticancer [22]. The therapeutic effect of these compounds could be attributed to their antioxidant activities [22].

Phenolic compounds are important phytochemicals with a variety of biological effects. According to, the presence of phenolics is related to the antioxidant activity of coconut water [22]. Phenolic acid has been shown to have a variety of therapeutic effects in the treatment of diseases such as diabetes, cancer, neurodegenerative, cardiovascular, and inflammatory disorders [20].

Flavonoids have been shown to have antibacterial, cytotoxic, anti-inflammatory, and anti-tumour properties, but their ability to function as potent antioxidants that protect the human body from free radicals and reactive oxygen species is the best-known attribute of every category of flavonoids [23]. This is in line with the findings of on Cocos nucifera tender coconut water [22]. Flavonoids such as flavone C-glycoside, kakonein, and caesalpin P increase pancreatic islet cell function and have anti-diabetic properties [20].

The tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours among other uses as reported by [24]. In clinical terms, all forms of tannins may participate in the management of glucose level in blood. Tannin has been shown to stimulate the receptor cells to utilize carbohydrate [20].

Terpenoids have anti-inflammatory activity and other pharmaceutical functions [25].

Cardiac glycosides are plant secondary metabolites that have a glycoside unit and act on the contractile action of the cardiac muscle. Their effects range from antioxidation to aromatase inhibition [26].

The tender and mature coconut water of Cocos nucifera showed a significant ($p < 0.05$) concentration-dependent DPPH radical scav-

enging capacity. The antioxidants in the liquid were able to neutralize the free radical character of DPPH probably by transferring either electron or hydrogen atoms to DPPH, thereby changing the colour from purple to the yellow-coloured diphenyl picrylhydrazine [11]. This interaction depends on the structural conformation of the bioactive compounds present in the plant among which the hydroxyl groups of flavonoids and phenolic are highly favourable [27]. The observed potent DPPH radical-scavenging capacity of the tender and mature coconut water corroborated with the concentration of the standard (ascorbic acid) with a positive correlation coefficient (R2) of 0.531, 0.108 and 0.901 respectively. There exist also significant ($p < 0.05$) difference between DPPH scavenging capacities of the liquids and that of the standard (ascorbic acid) at the various determined concentrations. This result is in line with the use of plants as radical scavenger [28, 11]. reported that the coconut water obtained from green dwarf Cocos nucifera was found to be extremely effective in scavenging DPPH radicals and thus attributed the antidiabetic potential to the radical scavenging capacity. This may be attributed to the antidiabetic and antioxidant potentials of tender and mature coconut water of Cocos nucifera. found the radical scavenging activity of coconut water to be concentration dependent with a significant ($p < 0.05$) decrease from that of the ascorbic acid standard and concluded that Cocos nucifera is a potential source of antioxidants and thus could prevent many radical diseases [11]. This study also showed that tender coconut water has higher DPPH Scavenging capacity than mature coconut water with IC50 of 1.87 mg/ml and 2.49 mg/ml respectively.

Ferric ion reducing antioxidant power assay (FRAP) measures the reducing potential of the ferric cyanide complex (Fe3+) to the ferrous cyanide form (Fe2+) in the presence of antioxidants. The antioxidants in the liquid (tender and mature coconut water) were able to reduce ferric cyanide complex (Fe3+) to the ferrous cyanide form (Fe2+.The effect of the liquid on reducing Fe3+ into Fe2+, was measured by reducing potential of the ferric cyanide complex (Fe3+) to the ferrous cyanide form (Fe2+) [29]. There exist no significant ($p > 0.05$) difference between the scavenging potential of the liquid and the ascorbic acid (standard) at the various determined concentrations. However, the liquid, tender and mature coconut water of Cocos nucifera was found to have a higher reducing activity (IC50 = 92.56 mg/ml and 137.39 mg/ml) respectively when compared to the standard ascorbic acid (IC50 = 223.79 mg/ml). This agrees with the report that phenolic compounds have redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers [14]. It has been reported that the reducing power of substances is probably because of their hydrogen-donating ability. Tender and mature coconut water might therefore, contain high level of reluctances than that of the standard (ascorbic acid) [30].

Phosphomolybdenum assay (PM) is based on the reduction of Phosphate-Mo (VI) to Phosphate Mo (V) by the liquid (tender and mature coconut water) and subsequent formation of a bluish green colored phosphate-Mo (V) complex at acid pH [31]. The results showed that tender and matured coconut water from Cocos nucifera could reduce Phosphate-Mo (VI) to Phosphate Mo (V) in a concentration-dependent mode with no significant difference ($p > 0.05$) from ascorbic acid (standard) with a good positive correlation coefficient (R2) of 0.93, 0.693, and 0.311 and (IC50 = 39.56, 48.39, and 19.89 mg/ml) respectively. The findings of this investigation corroborate the findings of [28].

The tender and mature coconut water of the Cocos nucifera fruit was shown to be nontoxic up to 5ml/kg body weight in an acute toxicity (LD50) test. This supports previous research by indicating that both tender and mature coconut water are safe for human and animal consumption [6].

The results revealed that there was a significance ($p < 0.05$) decrease in the amount of glucose present in the treated groups when compared to the untreated. The increase in blood glucose level in the untreated could be as a result of oxidative stress [32]. Insulin resistance, B-cell dysfunction, poor glucose tolerance, and type 2 diabetes mellitus have all been linked to oxidative stress [33]. The considerable (p0.05) drop in blood glucose levels in the test groups implies that the liquid includes antioxidants that may have countered oxidative stress in the experimental animals. An earlier study found that the antioxidant content of coconut water (mature coconut) reduces blood glucose levels in a synergistic way [6]. This finding suggests that tender and mature coconut water from the Cocos nucifera fruit have powerful antihyperglycemic properties and might thus be useful as a source of glucose.

Lipid peroxidation marker expressed as malondialdehyde (MDA) is mostly used to estimate the peroxidation processes. This aldehyde is produced by the radical breakdown of hydroperoxides resulting from poly unsaturated fatty acid peroxidation (PUFAs) containing at least two double bonds [9]. When compared to the untreated group, the MDA level in the treated group decreased significantly (p 0.05). The reduction in MDA levels observed in this study demonstrated that the liquid (tender and mature coconut water) could inhibit lipid peroxidation in the treated animals, demonstrating the liquid's antioxidant capacity. The current study's findings of increased lipid peroxidation during diabetes could be due to diabetes' inefficient antioxidant system. It's possible that the increased production of free radicals from glucose autoxidation, the polyol pathway, and non-enzymatic protein glycation is due to persistent hyperglycaemia [9].

Superoxide dismutase (SOD) is one of the most effective intracellular enzymatic antioxidants and it act as the first line defence system against ROS which scavenges superoxide radicals to dioxygen and hydrogen peroxide (H2O2): $O2^- + O2^- + 2H^+ \square H2O2 + O2$ [34]. The role of SOD in combating free radicals generated during infection could account for the decrease in SOD levels in the treated group. SOD levels were restored following treatment with the liquid. In the decomposition of intracellular hydrogen peroxide, catalase is a necessary enzyme [35]. The level of catalase in the treated animals was found to be higher than in the untreated animals in this study. The fact that the plant extract was able to

restore the level of catalase used to breakdown hydrogen peroxide was demonstrated by the fact that the animals' catalase levels increased. The capacity of antioxidant enzymes to scavenge ROS and hence prevent additional damage to membrane lipids might be linked to their restoration in levels. Therefore, the antioxidant properties of the liquid may have resulted in the recoupment in the activities of the enzymic antioxidants (SOD and catalase). Glutathione is one of the most important antioxidants that play a vital role in maintaining the cells redox state, since the thiol group in its cysteine is a reducing agent and can be reversibly oxidized and reduced [36]. The increase in the level of GSH in the treated group compared to the untreated animals showed that the liquid boosts the antioxidant system of the host organism. Vitamin C primarily functions as an intracellular and extracellular aqueous-phase antioxidant by scavenging oxygen free radicals. It converts vitamin E free radicals back to vitamin E. Its plasma levels have been shown to decrease with age [36]. Vitamin E which is a fat-soluble vitamin showed an increase in the level in the treated group compared to the untreated animals giving credence that the liquid boosts the antioxidant system of the host organism. The increase in level of vitamin observed in this study shows that the liquid was able to inhibit lipid peroxidation since Vitamin E (α tocopherol) is the most important lipid-soluble antioxidant and protects cell membranes against oxidation by reacting with the lipid radicals produced in the lipid peroxidation chain reaction and removing the free radical intermediates [35].

Conclusion

The results obtained from this study showed that tender and matured coconut water of Cocos nucifera fruit can be used for the management of diabetes in herbal medicine. This gives scientific evidence to the claims in different parts of the country that the fruit is used in the management of diabetes. These effects could be mainly attributed to its antioxidant properties as shown by impact of the liquid (tender and mature coconut water) on lipid peroxidation along with enhancement of antioxidant defence systems. The antioxidative property of the liquid certainly is due to its phytochemical constituents. The efficacy of tender and mature coconut water of *Cocos nucifera* are highly correlated and are safe for human consumption.

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