IVER, RENAL, AND ANTI-OXIDANT FUNCTIONAL BIOMARKERS CAN IMPROVE IN DOSE-DEPENDENT ADMINISTRATION OF FRESH COCONUT OIL

Abstract:

Overtime, Oxidative stress has been implicated in the progression of diabetes mellitus (DM) and its related disorders. To this point, several studies posit that antioxidant constituents of virgin coconut oil among others might have a helpful effect in ameliorating the disease. In this study, the impact(s) of ingestion of fresh coconut oil (FCO) on the liver, kidney, and anti-oxidant biomarkers was investigated in alloxan-induced diabetic Sprague Dawley. Ninety-eight (98) albino rats (100 - I50g) were randomly divided into two (2) units of forty-nine (49) rats each; with each unit subdivided into seven (7) groups of seven (7) animals each. At induction of diabetes mellitus (DM) in subgroups 2, 3, 4, 5, 6, 7 of unit 1 and B, C, D, E, F and G of Unit 2, rats in the 1 and A subgroups were left untouched to serve as control. Whereas unit 1 (treated for 2 weeks), subgroups 2-7 respectively received nothing (after DM confirmation), nothing (after DM confirmation), 7.5mg/kg of FCO, 10mg/kg of FCO, 7.5mg/kg of FCO plus Vitamin E, 10mg/kg of FCO plus Vitamin E, and only Vitamin E; Unit 2 animals (treated for 4 weeks) were given untreated (after confirming diabetes), 7.5mg/kg of FCO, 10mg/kg of FCO, 7.5mg/kg of FCO + Vitamin E, 10mg/kg of FCO and Vitamin E, and Vitamin E respectively for B-G subgroups. Following administration of test substance, serum samples were then collected from animals for biochemical analysis of liver enzymes, renal biomarkers and antioxidants enzymes. One way analysis of variance (ANOVA) proved that liver enzymes were significantly ($p < 0.05$) reduced, while antioxidant enzymes (SOD and CAT) were significantly increased ($p < 0.05$), the electrolytes levels, renal biomarkers (urea and creatinine) were insignificant. Also, changes recorded after four weeks followed the same pattern, showing that dietary factor-Vitamin E, modulates the effect of FCO. From this result, it is implied that FCO significantly improved metabolic parameters especially with significant reduction in oxidative stress in Type 1 diabetes mellitus

Keywords: Anti-oxidant, Biomarkers, Diabetes

INTRODUCTION

Even though there has been little or no scientific information on the mechanism of actions of some natural products and plants extracts[1], most have however been shown to pose anti-diabetic tendencies. Coconut oil for instance, has been renowned throughout history for its medicinal and nutritional value. In recent years, various experiments have been conducted

to show its biological effect(s). It has been shown to limit the activities of microbes and virus[2], enhance thyroid function and weight loss[3], diminish the low density lipoprotein (LDL) concentration, plus increase plasma and tissue levels of high density lipoprotein (HDL) – cholesterol[4&5].

The health promotional abilities and possible mechanisms of action of this oil has been shown by various researchers[6]. A group of researchers suggested that it reduces oxidative stress by boosting the antioxidant defence system, scavenging free radicals and reducing lipid peroxidation; Another independent study suggested that the oxidative stress linked with diabetes mellitus can be possibly reduced by the administration of fresh coconut oil, and thus improve metabolic activities in the disease[6]. Iranloye *et al*., (2013) reported that virgin coconut oil (VCO) causes a hypoglycaemic action by enhancing insulin secretion. They also showed the oxidative stress ameliorating effect of this oil on induced in type I (alloxan-induced) diabetic male rats[6&7].

These proven abilities of the oil in promoting some of the health conditions could be due to its phytochemical constituents like polyphenols and vitamin E, which can boost the antioxidant defence structure[5] and also, its medium chain fatty acids and unsaponifiable constituents. In recent times, great attention is being drawn to fresh coconut oil (FCO) as it is believed to be more beneficial than copra oil due to its method of extraction that makes it retain more of its natural active components[6]. The extraction of FCO from the fresh endosperm of coconut is thought to be more beneficial than usually prepared copra oil because its mode of extraction retains more biologically active components such as alpha tocopherol (vitamin E) and polyphenols[6]. Thus, this study was necessitated to determine the effect(s) of fresh coconut oil on the live, kidney, and antioxidant metabolites in type I diabetes in Sprague Dawley Rats.

Aim of Study

Study aimed at investigating the effect(s) of fresh coconut oil (FCO) on biomarkers of liver, kidney, and antioxidant function in type I diabetes in sprague dawley rats. Study was specifically geared towards:

i. ascertaining the effect(s) of FCO on Antioxidant Enzymes (Superoxide dismutase, Catalase and Malonaldehyde)

ii. Investigating the effect(s) of fresh Coconut oil on Lipid profile (Total Cholesterol, Triglyceride, HDL and LDL).

iii. assessing the effect(s) of fresh Coconut oil on liver enzymes (Alkaline Phosphatase, Alanine Aminotransferase, Aspartate Amino Transferase, Lactate Dehydrogenase, Gamma glutathione Transferase)

Methodology

Scope of Study

Study was best suited for rats as the invasive nature would be inappropriate in humans. It was limited to the effects of the ingestion of FCO on some metabolic functions specifically serum electrolyte levels, liver enzymes, Oxidative stress status - antioxidant enzymes and lipid profile, using Sprague Dawley Rats as experimental model

Study Design

Ninety-eight (98) rats, weighing between 100 - I50g and bred in the Animal house of the Faculty of Basic Medical Sciences of Delta State University, Abraka were used for this study. Acclimatization was at the Animal house of the Department of Physiology of Delta State University, Abraka. Animals were then divided into two (2) units of 49 rats each. Each unit was further divided into Seven (7) groups, each containing seven animals (n = 7).

UNIT 1

Group 1: Control (C): Normal rats fed with rat chow and drinking water.

Group 2: Diabetic rats untreated (DUT)

Group 3: Diabetic rats treated with 7.5mg/kg body weight of fresh coconut oil ($DT_{7.5}$) for two (2) weeks

Group 4: Diabetic rats treated with l0mg/kg body weight of fresh coconut oil (DT_{10}) for two (2) weeks

Group 5: Diabetic rats treated with 7.5mg/kg body weight of fresh coconut oil ($DT_{7.5}$) + Vitamin E for two (2) weeks

Group 6: Diabetic rats treated with 10mg/kg body weight of fresh coconut oil ($DT_{7.5}$) + Vitamin E for two (2) weeks

Group 7: Diabetic rats treated with Vitamin E for two (2) weeks

UNIT 2

Group A: Control (C) Normal rats fed with rat chow and drinking water.

Group B: Diabetic rats untreated (DUT) for four (4) weeks

Group C: Diabetic rats treated with 7.5mg/kg body weight of fresh coconut oil ($DT_{7.5}$) for four (4) weeks

Group D: Diabetic rats treated with l0mg/kg body weight of fresh coconut oil (DT_{10}) for four (4) weeks

Group E: Diabetic rats treated with 7.5mg/kg body weight of fresh coconut oil ($DT_{7.5}$) + Vitamin E for four (4) weeks

Group F: Diabetic rats treated with 10mg/kg body weight of fresh coconut oil ($DT_{7.5}$) + Vitamin E for four (4) weeks

Group G: Diabetic treated with Vitamin E for four (4) weeks

Materials

Used materials include; wire-guaze cages, normal rat Chow and clean water. A well-ventilated animal house to allow for homeostatic conditions

Procedure

Ethical Clearance

Ethical clearance was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State, with rules for handle of laboratory animals strictly adhered to.

Preparation of Fresh Coconut Oil (FCO)

Matured coco-nuts were procured and its oil (FCO) was extracted using the wet extraction method described by Nevin and Rajamohan (2006) and Dosumu *et al.,* (2010). The solid endosperm was then crushed into thick slurry[8&9]. About 500 millilitre (ml) of water was added to the thick slurry obtained by squeezing through a fine filter to obtain the milk. The resulting coconut milk was allowed to settle for about twenty four (24) hours, allowing for sedimentation to take occur. This lead to separation of its emulsion (Demulsification), producing different layers of an aqueous phase (water) at the bottom, an emulsion (cream) formed the middle layer and oil on top of the emulsion. The oil on top was taken and heated for about five (5) minutes to evaporate the moisture. Obtained coconut oil was filtered thoroughly through a fine filter and stored at room temperature for use in the experiment.

Sample Collection

At the end of the 4 weeks period administering test substance, blood samples were collected from the orbital sinus of all animals through ocular puncture, following which they were sacrificed by cervical dislocation, with selected viscerals harvested for a separate study. Serum was separated by centrifuging at 6000rpm for 15 mm. various biochemical analyses were thereafter conducted on obtained blood samples.

Induction of Diabetes Mellitus

After two (2) weeks of acclimatization, Alloxan monohydrate was used to cause type I diabetes in experimental animals. Intraperitoneal administration of 100mg/kg body weight of Alloxan monohydrate was administered once. A mild pressure was applied at the spot of injection to enhance absorption. After 3 days of administration, fasting blood glucose level of

rats was measured. Rats with fasting blood glucose level above 200mg/dl were considered diabetic.

Biochemical Assays

Determination of antioxidant enzymes and lipid peroxidation

At the end of the four (4) week period of experimentation, animals were euthanized via cervical dislocation, with liver harvested, washed, crushed and homogenized in KCl solution. The homogenate was diluted and centrifuged, while supernatant was decanted and examined for various antioxidant enzymes as follows:

Superoxide Dismutase (SOD) Assay

Superoxide dismutase enzyme activity was determined according to the method of Soon and Tan (2002)[10]. It was measured by its ability to inhibit auto-oxidation of epinephrine. The assay was performed in 3.0ml of 50mM sodium bicarbonate buffer (in 2 different test tubes) to which 0.02m1 of extract was added. 0.03m1 of epinephrine stock solution was then added to the above before taking absorbance readings at 480nm for 3 – 5mins. A blank bereft of the sample was used for circumstantial correction. Enzyme activities were expressed as SOD units, where one unit of SOD is defined as the quantity of enzyme needed to inhibit fifty percent (50%) epinephrine per minute, per milligram of protein at 25°C and pH 7.8.

atalase (CAT) Assay

Activities of catalase enzyme was analysed according to the method of Soon and Tan (2002) who measured the initial rate of H_2O_2 (50mM) decomposition at 240nm with the results expressed in units/mg protein, where one unit is the amount of enzyme that hydrolyses 1 µmol of H_2O_2 per minute and per milligram of protein at 30°C and pH 8.0. To 0.3ml (300 ul) of extract sample 1.8 of 30 mM H_2O_2 was added. Phosphate buffer was used as the blank and their absorbance reading were taken at 240 nm at 60s intervals for 5mins.

Reduced Glutathione Assay

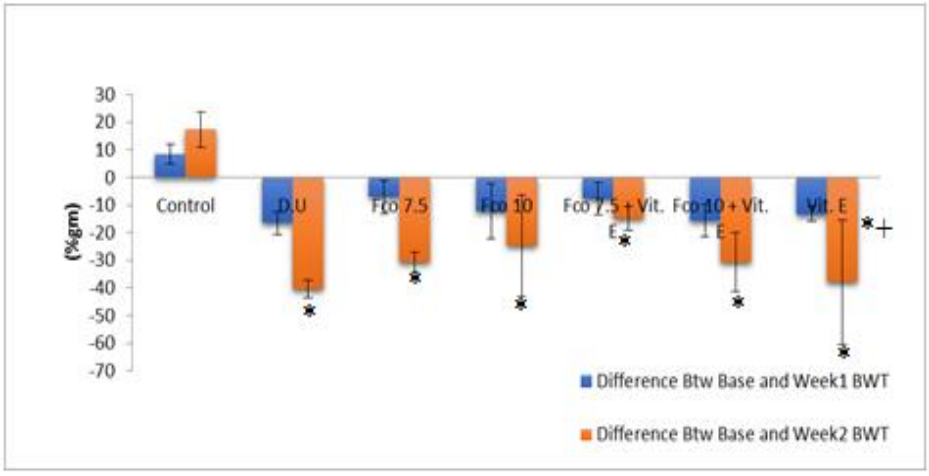
This was determined using 5, 5-dithio-bis-2-nitrobenzoic acid (DTNB) whose chemical formula is C and Tris-EDTA buffer with the absorbance being read at 412 nm (Soon and Tan, 2002). 100µl sample was added to 1ml of 0.2M Tris-EDTA buffer, pH 8.2. 0.9ml of 20mM EDTA, pH 4.7 was added 20ul of 10mM DTNB was added and the sample was allowed to incubate at room temperature. The mixture was centrifuged and the absorbance of the supernatant was read against distilled water at 412 nm. Calculation was made using: GSH = OD/ X V/v, where OD = absorbance; = extinction coefficient; V = total volume of reaction mixture; and v = volume of sample in reaction mixture.

Statistical Analysis

With data represented as mean standard deviation, Statistical analysis was done using One-Way Analysis of Variance (ANOVA). Statistics was carried out with SPSS 22 software. A p-level less than 0.05 was considered as statistically significant

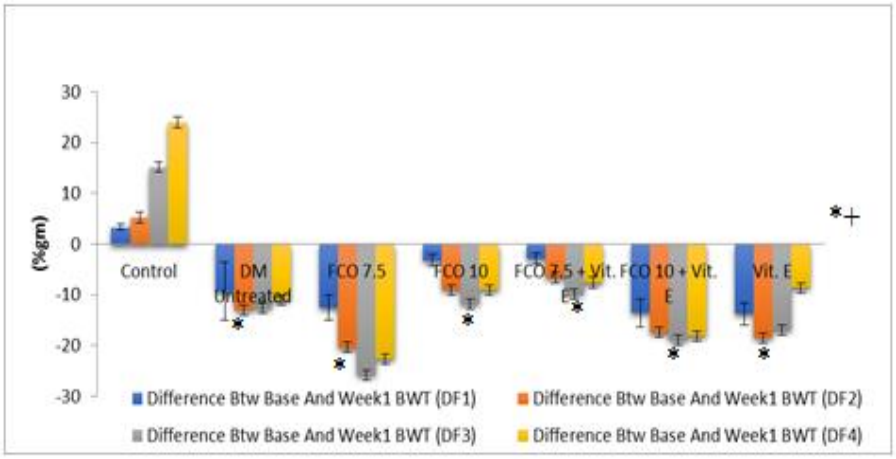
Results

CHART 1: Body weight changes after 2 weeks of treatment (Unit 1 Experiment)



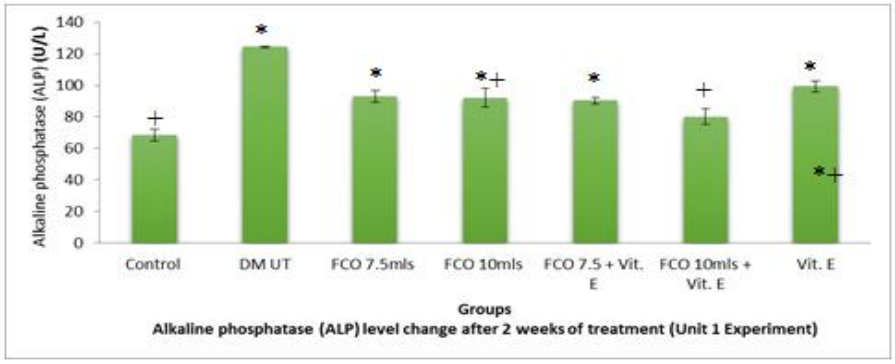
Values are expressed as mean ± S.E.M, n=5. Mean differences was compared using both one way analysis of Variance and Student-T test, and significant values were determined at P≤0.05 level and designated as (*) when compared with control and (+)when compared with diabetic untreated.

CHART 2: Graphical representation of body weight changes after 4 weeks of treatment (Unit 2 Experiment)



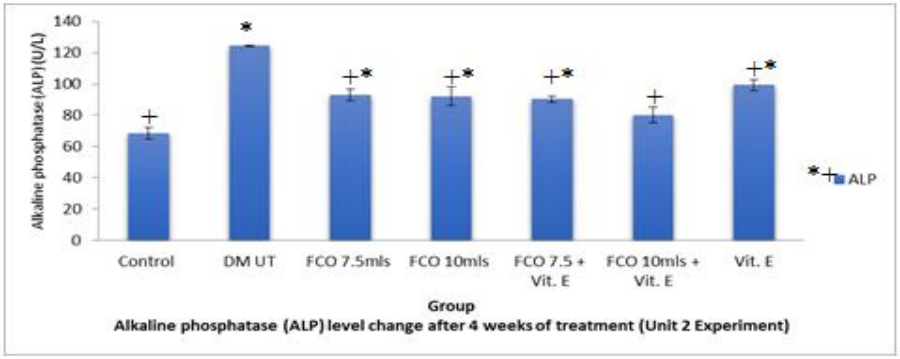
Values are expressed as mean ± S.E.M, n=5. Mean differences was compared using both one way analysis of Variance and Student-T test, and significant values were determined at P≤0.05 level and designated as (*) when compared with control and (+)when compared with diabetic untreated.

CHART 3: Alkaline phosphatase (ALP) changes after 2 weeks of treatment (Units 1 and 2)



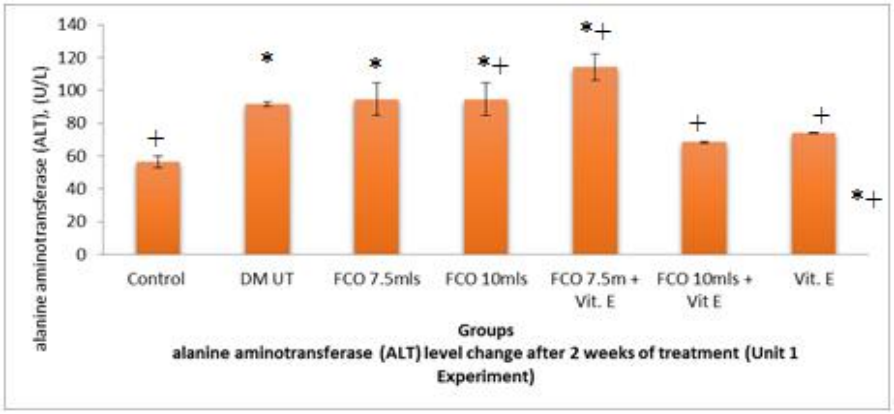
Values are expressed as mean ± S.E.M, n=5. Mean differences was compared using both one way analysis of Variance and Student-T test, and significant values were determined at P≤0.05 level and designated as (*) when compared with control and (+)when compared with diabetic untreated.

CHART 4: **Alkaline Phosphatase (ALP) changes after 4 weeks of treatment**



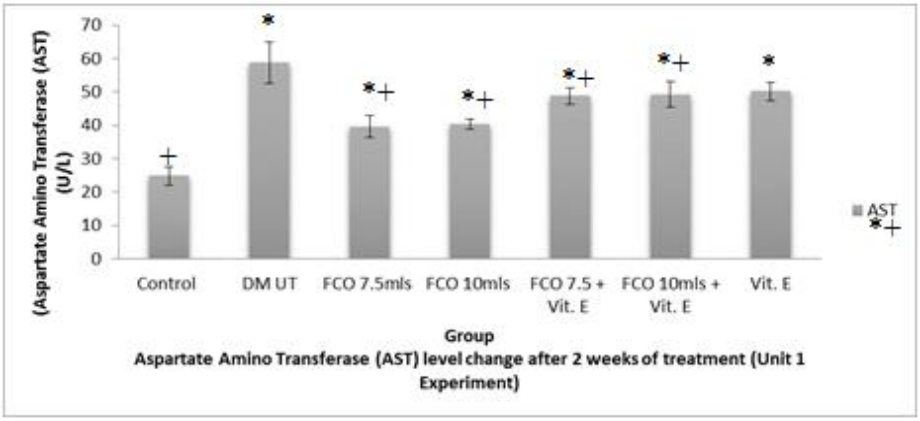
Values are expressed as mean ± S.E.M, n=5. Mean differences was compared using both one way analysis of Variance and Student-T test, and significant values were determined at P≤0.05 level and designated as (*) when compared with control and (+)when compared with diabetic untreated.

CHART 5: **Alanine Aminotransferase (ALT) changes after 2 weeks of treatment**



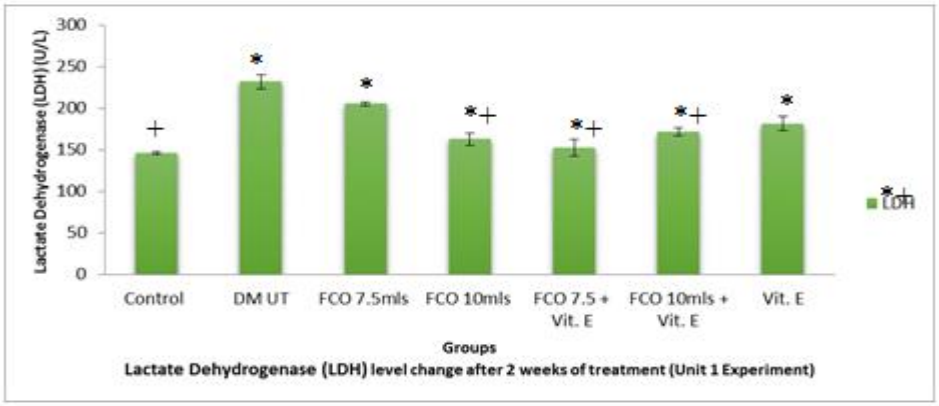
Values are expressed as mean ± S.E.M, n=5. Mean differences was compared using both one way analysis of Variance and Student-T test, and significant values were determined at P≤0.05 level and designated as (*) when compared with control and (+)when compared with diabetic untreated.

CHART 6: Aspartate Amino Transferase (AST) level changes of two weeks treatment in alloxan-induced diabetic rats



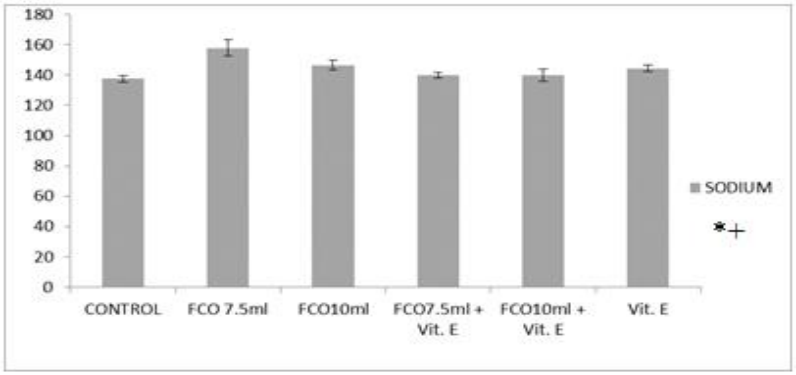
Values are expressed as mean ± S.E.M, n=5. Mean differences was compared using both one way analysis of Variance and Student-T test, and significant values were determined at P≤0.05 level and designated as (*) when compared with control and (+)when compared with diabetic untreated.

CHART 7: Effect of FCO extract on Lactate Dehydrogenase (LDH) level changes of four weeks treatment in alloxan-induced diabetic rats.



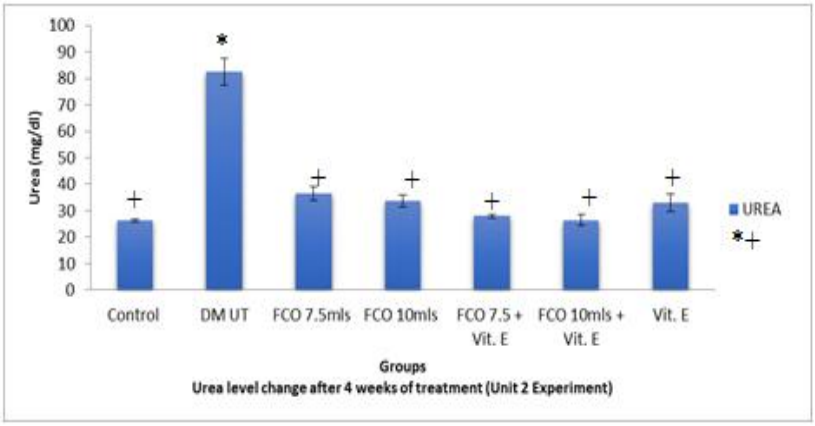
Values are expressed as mean ± S.E.M, n=5. Mean differences was compared using both one way analysis of Variance and Student-T test, and significant values were determined at P≤0.05 level and designated as (*) when compared with control and (+)when compared with diabetic untreated.

CHART 8: Effect of FCO extract on Sodium level changes of four weeks treatment in alloxan-induced diabetic rats.



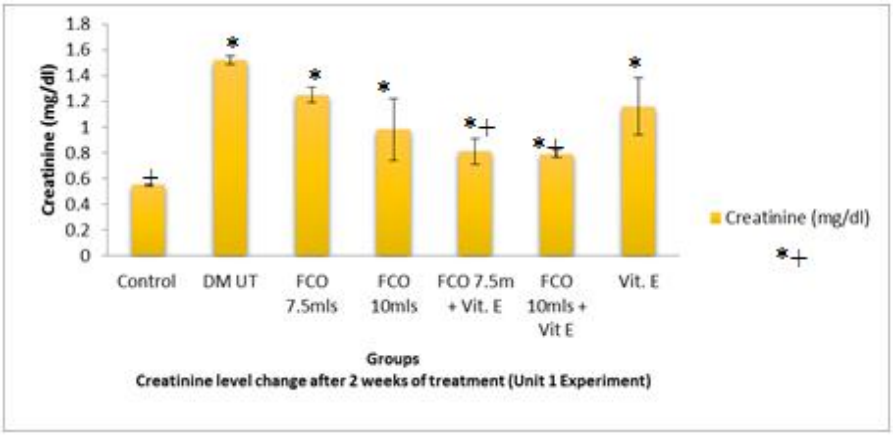
Values are expressed as mean ± S.E.M, n=5. Mean differences was compared using both one way analysis of Variance and Student-T test, and significant values were determined at P≤0.05 level and designated as (*) when compared with control and (+)when compared with diabetic untreated.

CHART 9: Effect of FCO extract on Urea level changes of four weeks treatment in alloxan-induced diabetic rats.



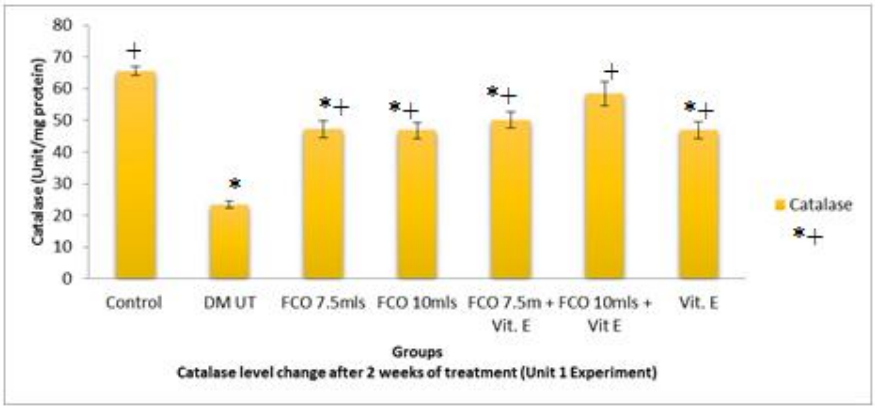
Values are expressed as mean ± S.E.M, n=5. Mean differences was compared using both one way analysis of Variance and Student-T test, and significant values were determined at P≤0.05 level and designated as (*) when compared with control and (+)when compared with diabetic untreated.

CHART 10: Effect of FCO extract on Creatinine level changes of two weeks treatment in alloxan-induced diabetic rats.



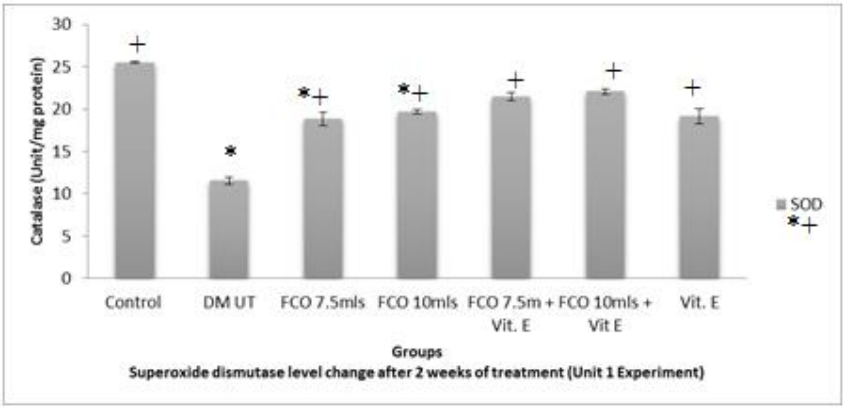
Values are expressed as mean ± S.E.M, n=5. Mean differences was compared using both one way analysis of Variance and Student-T test, and significant values were determined at P≤0.05 level and designated as (*) when compared with control and (+)when compared with diabetic untreated.

CHART 11: Effect of FCO extract on Catalase level changes of two weeks treatment in alloxan-induced diabetic rats.



Values are expressed as mean ± S.E.M, n=5. Mean differences was compared using both one way analysis of Variance and Student-T test, and significant values were determined at P≤0.05 level and designated as (*) when compared with control and (+)when compared with diabetic untreated.

CHART 12: Effect of FCO extract on Superoxide Dismutase (SOD) level changes of two weeks treatment in alloxan-induced diabetic rats.



Values are expressed as mean ± S.E.M, n=5. Mean differences was compared using both one way analysis of Variance and Student-T test, and significant values were determined at P≤0.05 level and designated as (*) when compared with control and (+)when compared with diabetic untreated.

Discussion

Medicinal plants are commonly used by the inhabitants of developing countries as an alternative to orthodox therapy. In Africa alone, hundreds of plants are used traditionally for the management and/or control of diabetes mellitus. Regrettably, only a few of such African medicinal plants have received scientific examination. Coconut has been listed by various authors as a potent medicinal nut.

The oil from this nut has been widely used throughout history for its medicinal value and has served man as important food for thousands of years[11&12]. The proven abilities of coconut oil in promoting health could be due to phytochemical constituents like polyphenols and vitamin E which can boost the antioxidant defence structure[5] and also, its medium chain fatty acids and unsaponifiable constituents. It contains a mixture of triglycerides consisting only of short and medium chain saturated fatty acids ninety-two percent (92%) and unsaturated fatty acids eight percent (8%) (Dayrit, 2003; Reynolds, 1982). Chemical analysis of Coconut water showed that it also contains L-arginine (5.85%), magnesium (0.42%), ascorbic acid (0.45%), potassium (7.71%), manganese (0.084%), calcium (1.32), total proteins (13.6%) etc. Among these, L-arginine noted to be the main bioactive component, which has been shown to have lots of beneficial antagonizing effects on diabetes[13]. The potential benefits of FCO in preventing or ameliorating different biological conditions due to its active polyphenol components has been demonstrated[4]. Below is an analysis of the propable explanations and theoretical structure of the findings from this study.

Chat 1(above) shows effect of FCO and Vitamin E on body weight of Wister rats after two weeks of treatment. Result shows significant loss in body weight obtained between first week and week 2, with a decrease in body weight. This decrease was significant as compared

with control, implying that treatment with FCO and Vitamin E at all doses causes significant decrease in body weight within two weeks of treatment in alloxan-induced diabetes.

Chat 2(above) shows effect of FCO and Vitamin E on body weight (g) of Wister rats after four weeks of treatment. Compared with control, result shows significant loss in body weight (g) of all experimental groups. This implies that treatment with FCO at all doses with Vit. E and separately does not improve body weight (g) in alloxan induced diabetes.

Chats 3 and 4 show effect of FCO and Vit. E on the liver's Alkaline Phosphatase (ALP) level after two weeks of treatment. Result shows a significantly elevated ALP level in all experimental groups except FCO High dose combined with Vit.E (FCO 10mls +Vit.E) group. Furthermore, when experimental groups were compared with diabetes untreated, there was significantly decrease in ALP level in FCO separate and combined (FCO +Vit. E) high dose groups, while others showed insignificant effect. This implies that treatment with FCO at High dose separately and combined with Vit. E significantly improved ALP level while FCO low dose and Vit. E separately does not in diabetes.

Chat 5 shows effect of FCO and Vit. E on ALT level. Result shows significantly elevated ALT level in all experimental groups except Vit. E separate and FCO High dose combined with Vit.E (FCO 10mls +Vit.E). Moreover, when experimental groups were compared with diabetes untreated there was significantly decreased ALT level in all experimental groups. This implies that treatment with FCO at all doses and combined with Vit. E separately improves ALT level in diabetes.

Chat 6 shows effect of FCO and Vit. E on AST level of Sprague Dawley Rats after two weeks of treatment. Results show significantly difference in AST level in all experimental groups when compared with control. However, when experimental groups were compared with diabetes untreated there was significantly decreased AST level in all experimental groups except in groups treated with Vit. E alone. This implies that treatment with FCO at all doses and also when combined with Vit E. significantly improves AST level in diabetes.

Chat 7 shows effect of FCO and Vit. E on Lactate Dehydrogenase (LDH) level. Result shows significant difference in LDH levels for all experimental groups. In addition, when experimental groups were compared with diabetes untreated, there was significantly decrease in LDH level in all experimental groups except at Low dose FCO and Vit E alone. This implies that treatment with FCO at High dose separately and in combination with Vit. E at all doses significantly improves LDH level in alloxan induced diabetes.

Chat 8 shows effect of FCO and Vit. E on Sodium level, of Sprague Dawley Rats after four weeks of treatment. Results showed no significant change in Sodium level in all experimental groups when compared with control. In addition there was no significant change in Sodium level in all experimental groups when compared with diabetes untreated group. This implies that treatment with FCO separately and combined with Vit. E do not improve Sodium level in diabetes.

Chart 10 shows the effect of FCO and Vit. E on Urea level, of Sprague Dawley Rats after four weeks of treatment. ResultS showed that Urea level is not significantly affected in all experimental groups when compared with control. Moreover, when experimental groups were compared with diabetes untreated, there was significantly decreased urea level in all experimental groups. This implies that treatment with FCO at all doses and Vit. E separately and combined significantly improves Urea level in diabetes.

Chart 11 shows effect of FCO on Catalase after two weeks of treatment. Result shows significantly increased Catalase level in all treated groups except high dose FCO combined with Vit. E when compared with control. Moreover, when experimental groups were compared with diabetes untreated, there was significantly increased Catalase level in all experimental groups. This implies that treatment with FCO at all doses and in combination with Vit. E significantly improves Catalase level in diabetes. After weeks of treatment, Catalase level was seen to have insignificantly been affected in all experimental groups compared to control. Moreover, when experimental groups were compared with diabetes untreated, there was significantly increased Catalase level in all experimental groups. This implies that treatment with FCO at all doses and in combination with Vit. E significantly improves Catalase level in diabetes.

Chart 12 shows effect of FCO and Vit. E on SOD level after two weeks of treatment. Results show significantly decreased SOD level in FCO separate doses while combined FCO and Vit. E experimental groups were not significantly affected as compared with control. Moreover, when experimental groups were compared with diabetes untreated, there was significantly elevated SOD level in all experimental groups. This implies that treatment with FCO at all doses and Vit. E separately and combined significantly improves SOD level in diabetes. For unit 2 animals, result shows significant effect on SOD level in all experimental groups as compared with control. However, when experimental groups were compared with diabetes untreated, there was significantly increased SOD level in all experimental groups. This implies that treatment with FCO at all doses with Vit. E separately and combined, significantly improves SOD level in diabetes.

Significance of Study

Study will play significant roles in the recent drive to investigate human metabolic functions with regard to diet and lifestyle, and thus provide general knowledge towards the dangers/benefits of natural products. The study will also establish mechanism on the ameliorative functions of fresh Coconut oil in the different parameters examined. Data generated from it will provide information that will aid proper delivery of health services by dieticians and other related practitioners.

Conclusion

Treatment of diabetic rats with FCO significantly improved metabolic outcomes in diabetic Sprague Dawley Rats. In this study, FCO treatment was seen to rival the beneficial effects of vitamin E in almost all parameters measured, suggesting that FCO and Vitamin E treatment have similar anti-oxidant activities. More so, FCO treatments showed a dose-dependent effect on most parameters measured, with more significant outcomes in higher dose. These discoveries were orchestrated by a cascade of events within various mechanisms germane to physiological outcome

Recommendations

We recommend the frequent intake of FCO as it improve antioxidant enzyme activities, and causes a decrease in products of lipid peroxidation

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