

Mitigations in Testicular, Hepatic, and Pancreatic Histo-morphology in Alloxan-induced Diabetic Sprague Rats Treated with Fresh Coconut Oil and Vitamin E

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Authors' contributions

This work was carried out in collaboration between all authors. Author EMA carried out the bench work. Author BCO performed the literature searches, wrote and monitored the first draft of the manuscript. Authors AON and JCI managed and supervised the experimental protocol. All authors read and approved the final manuscript.

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ABSTRACT

Diabetes Mellitus (DM) is a condition in which the pancreas produces insufficient amounts of insulin, or in which the body's cells fail to respond appropriately to available insulin. In diabetics, glucose levels build up in the blood and urine, causing excessive urination, thirst, hunger, and problems with fat and protein metabolism. Studies have shown that antioxidant constituents of

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virgin coconut oil might be helpful in ameliorating DM. The present study investigated the lessening effect(s) of fresh coconut oil (FCO) intake on the deterioration of liver and pancreatic tissues in alloxan-induced diabetic Sprague Dawley rats. Ninety-eight (98) albino rats (100 - 150g) 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 1 and A subgroups were left untouched to serve as a control. Whereas, unit 1 (treated for 2 weeks), subgroups 2-7 respectively received nothing (after DM confirmation), nothing (after DM confirmation), 7.5 mg/kg of FCO, 10 mg/kg of FCO, 7.5 mg/kg of FCO plus Vitamin E, 10 mg/kg of FCO plus Vitamin E, and only Vitamin E; Unit 2 animals (treated for 4 weeks) were given untreated (after confirming diabetes), 7.5 mg/kg of FCO, 10 mg/kg of FCO, 7.5 mg/kg of FCO + Vitamin E, 10 mg/kg of FCO and Vitamin E, and Vitamin E respectively for B-G subgroups. Following administration of test substance, rats were euthanized by cervical dislocation, testes, pancreas and liver tissues harvested for histo-architectural analysis/changes. One way analysis of variance (ANOVA) showed that dietary factor-Vitamin E, significantly modulates the effect of FCO with significant improvement in histo-architecture in Type 1 diabetes mellitus.

Keywords: Diabetes mellitus; histo-architecture; fresh coconut oil.

1. INTRODUCTION

Widely distributed in tropical regions of the world, coconut is a common name for fruits of the tree of palm family [1]. It is Classified as *Cocos nucifera*, the coconut palm belongs to the family Arecaceae (formerly Palmae), and are used in vegetable oils and fats for cooking, food manufacture, drugs, soap making, specialized lubricating oils, and cosmetics [2,3].

Primary sources of vegetable oils are coconut, corn, cottonseed, oil palm, olive, peanut (groundnut or arachis oil), safflower, soybean, sunflower seed, and rapeseed. Most vegetable oils are rich in monounsaturated or polyunsaturated fatty acids, and nutrition experts consider them to be a more desirable dietary ingredient than saturated animal fats, such as butter or lard. Coconut oil consists of 92% saturated fatty acid and 8% unsaturated fatty acid. Most of the saturated fats are medium chain fatty acids having 10 to 12 carbon atoms [4].

Natural products and extracts of coconut oil, have been used and shown to have anti-diabetic properties although there has been little or no scientific information on the mechanism of actions of some of these plants [2]. Coconut oil has been renowned throughout history for its medicinal and nutritional value. More so, recent studies have shown that virgin coconut oil (VCO) has a varied degree of beneficial properties such as antiviral, antibacterial, anti-fungal, anti-inflammatory, antidiabetic, antiobesity, antiulcerogenic, analgesic and antipyretic, and antioxidant properties [5]. In a study, mature

coconut water was discovered to possess significant beneficial effects in diabetic rats and its effects were comparable to that of glibenclamide, a well-known antidiabetic drug [6].

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 per cent (92%) and unsaturated fatty acids eight percent (8%) [7]. 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. The potential benefits of FCO in preventing or ameliorating different biological conditions due to its active polyphenol components has been demonstrated [7].

1.1 Aim of Study

Study aimed at investigating the effect(s) of intake of fresh coconut oil (FCO) on the histology of the testes, liver, and pancreatic tissues in alloxan-induced diabetic Sprague Dawley rats. Study specifically:

- i. Examined the effect of fresh Coconut oil on the histology of the liver, testis and pancreas.

- ii. Evaluated the effects of fresh Coconut oil on Blood glucose level,
- iii. Assessed the effects of fresh Coconut oil on assessing the effect of Insulin level.

2. METHODOLOGY

2.1 Scope of Study

Study was limited to rats as the invasive nature would be inappropriate in humans. It was limited to the ascertaining the effect(s) of ingestion of FCO on some metabolic functions; specifically on blood glucose, insulin level, as well as on the histo-architecture of selected tissues, using Sprague Dawley Rats as an experimental model.

2.2 Procedure

2.2.1 Study design

Ninety-eight (98) rats, weighing between 100 - 150g and bred in the Animal house of the Faculty of Basic Medical Sciences of Delta State University, Abraka were used for this study. Following two weeks acclimatization period after purchase, animals were then divided into two (2) units of 49 rats each, with each unit subdivided 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 Untreated Rats (DUT)
- Group 3: Diabetic rats treated with 7.5 mg/kg body weight of fresh coconut oil (DT_{7.5}) for two (2) weeks
- Group 4: Diabetic rats treated with 10 mg/kg body weight of fresh coconut oil (DT₁₀) for two (2) weeks
- Group 5: Diabetic rats treated with 7.5 mg/kg body weight of fresh coconut oil (DT_{7.5}) + Vitamin E (50 mg/day) for two (2) weeks
- Group 6: Diabetic rats treated with 10 mg/kg body weight of fresh coconut oil (DT_{7.5}) + Vitamin E (50 mg/day) for two (2) weeks
- Group 7: Diabetic rats treated with Vitamin E (50 mg/day) 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 7.5 mg/kg body weight of fresh coconut oil (DT_{7.5}) for four (4) weeks
- Group D: Diabetic rats treated with 10 mg/kg body weight of fresh coconut oil (DT₁₀) for four (4) weeks
- Group E: Diabetic rats treated with 7.5 mg/kg body weight of fresh coconut oil (DT_{7.5}) + Vitamin E (50 mg/day) for four (4) weeks
- Group F: Diabetic rats treated with 10 mg/kg body weight of fresh coconut oil (DT_{7.5}) + Vitamin E (50 mg/day) for four (4) weeks
- Group G: Diabetic treated with Vitamin E (50 mg/day) for four (4) weeks.

2.2.2 LD₅₀ test

According to the American Society for Testing and Materials (1987), any chemical substance with LD₅₀ estimate greater than 3,000-5,000 mg/kg (Oral route) could be considered of low toxicity and safe. Based on this, 50 mg/kg body weight of FCO was used for the experiment.

2.3 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 the handle of laboratory animals strictly adhered to.

2.4 Preparation of Fresh Coconut Oil (FCO)

Matured coconuts were procured and its oil (FCO) was extracted using the wet extraction method described by Nevin and Rajamohan and Dosumu et al. [8-11]. The solid endosperm was then crushed into thick slurry. 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.

2.5 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 the testes, liver, and pancreas harvested for histological study. Serum was separated by centrifuging at 6000 rpm for 15 mm, and biochemical tests were conducted on obtained blood samples to obtain glucose level.

2.6 Induction of Diabetes Mellitus

At the end of the acclimatization period (two (2) weeks), type I diabetes was induced in experimental animals, using Alloxan monohydrate. Intraperitoneal administration of 100mg/kg body weight of Alloxan monohydrate was done 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 200 mg/dl were considered diabetic [12,13].

2.7 Measurement of Blood Glucose

Blood glucose level was measured with the One Touch Ultra test strips. Blood was obtained from the rats by severing the tip of their tail. The blood was dropped on the test strips already placed in a One (1) Touch Ultra Easy Glucometer (Lifescan Inc. Milpitas, USA). Blood glucose concentration was measured at the start of the experiment and every week for 4 weeks.

2.8 Measurement of Serum Insulin Level

Enzyme-Linked Immunosorbent Assay (ELISA, from Life scan Inc. Milpitas, USA) was used to measure the level of insulin in the serum sample obtained from the animal [14]. The reaction mixtures were incubated at room temperature for 2 hours after which the contents were removed from each well. The wells were washed with 300 μ L of diluted Wash Solution. After washing, 100 μ L of TMB-substrate each was added to the 3 microplate wells (Sample, Standard and Blank). The reaction mixture was incubated at room temperature for 15 minutes in the dark after which 100 μ L of Stop solution each was added to the 3 microplate wells. Insulin levels were measured reading the absorbance at 450 nm against Blank.

2.9 Histological Analysis of Pancreas, Liver and Testis

After harvesting the organ, tissues were placed inside well labelled tissue embedding cassette, and processed with a 24 hours automatic tissue processor. The tissue processor contained 12 beakers i.e. 10 glass beakers and 2 thermostatically controlled electric metal. Beaker 1 contained 10% formal saline; for complete fixation. Beaker 2-8 contained different ascending grades concentrations of dehydrating fluids (alcohol ranging from seventy percent (70%) alcohol to absolute (100%) alcohol (isopropyl alcohol). This helped to remove water from the tissue samples. Beaker 9-10 contained clearing agents (Xylene I and II) which completely clears the dehydrating agent off the tissue sample. Beaker 11-12 contained embedding agents, i.e. molten paraffin wax. After the tissues have been processed they were embedded using an automatic embedding centre containing paraffin wax. The paraffin wax which became solidified when cold formed a solid support medium for the tissue during sectioning. A microtome was used in cutting sections of pancreatic tissue. The sections cut were placed in a grease free slide which was then placed on a hot plate for 30 minutes in order for sections to adhere to the slides. Using the Haematoxylin and Eosin method, the section was dewaxed in xylene, taken to water using descending grades of alcohol i.e. from absolute alcohol to 95% alcohol, 70% alcohol and then water, stained in Haematoxylin for 10 mins, rinsed in water, differentiated in 1% acid alcohol and again, rinsed in water with counter stained in 1% Eosin for 2-5 mins. It was then dehydrated using ascending grades of alcohol (70% alcohol to 95% alcohol to absolute alcohol) and Cleared in xylene while mounted for view under the microscope [15].

2.10 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 < .05 was considered as statistically significant.

3. RESULTS

Results are presented as graph and plates below.

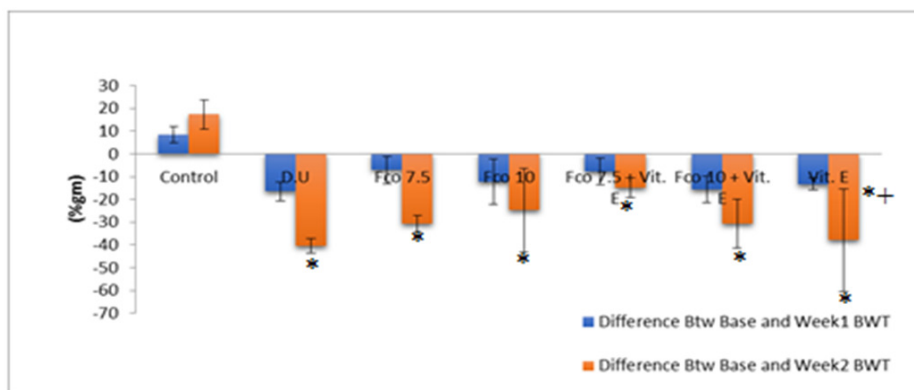


Chart 1. Body weight changes after 2 weeks of treatment (Unit 1 Experiment)

Values are expressed as mean \pm S.E.M, n=5. Mean differences was compared using the one way analysis of Variance (ANOVA). Significant values were determined at $P \leq 0.05$ level and designated as (*) when compared with control and (+) when compared with diabetic untreated

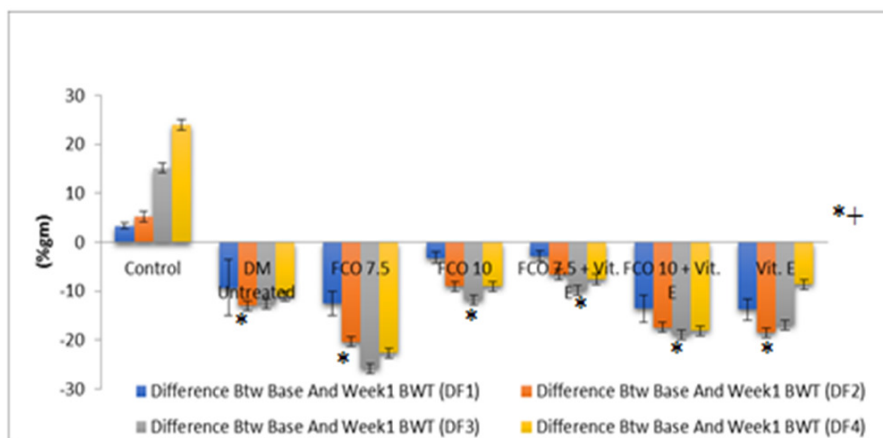


Chart 2. Body weight changes after 4 weeks of treatment (Unit 2 Experiment)

Values are expressed as mean \pm S.E.M, n=5. Mean differences was compared using the one way analysis of Variance (ANOVA) Significant values were determined at $P \leq 0.05$ level and designated as (*) when compared with control and (+) when compared with diabetic untreated

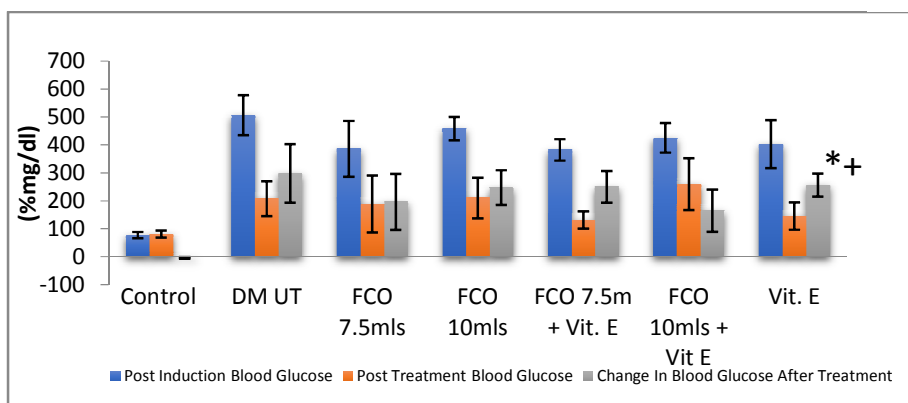


Chart 3. Changes in blood glucose after 2 weeks of treatment (Unit 1 Experiment)

Values are expressed as mean \pm S.E.M, n=5. Mean differences was compared using the one way analysis of Variance (ANOVA). Significant values were determined at $P \leq 0.05$ level and designated as (*) when compared with control and (+) when compared with diabetic untreated

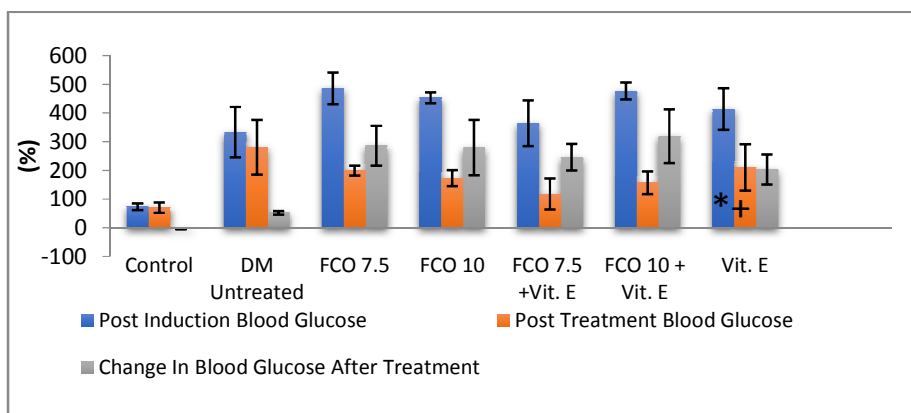


Chart 4. Changes in blood glucose after 4 weeks of treatment (Unit 2 Experiment)
 Values are expressed as mean \pm S.E.M, n=5. Mean differences was compared using the one way analysis of Variance (ANOVA). Significant values were determined at $P \leq 0.05$ level and designated as (*) when compared with control and (+) when compared with diabetic untreated.

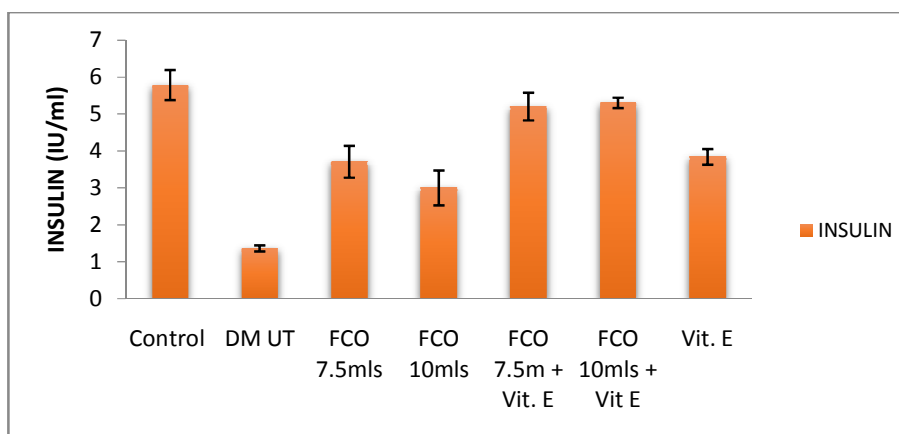


Chart 5. Insulin level change after 2 weeks of treatment (Unit 1 Experiment)
 Values are expressed as mean \pm S.E.M, n=5. Mean differences was compared using the one way analysis of Variance (ANOVA). Significant values were determined at $P \leq 0.05$ level and designated as (*) when compared with control and (+) when compared with diabetic untreated.

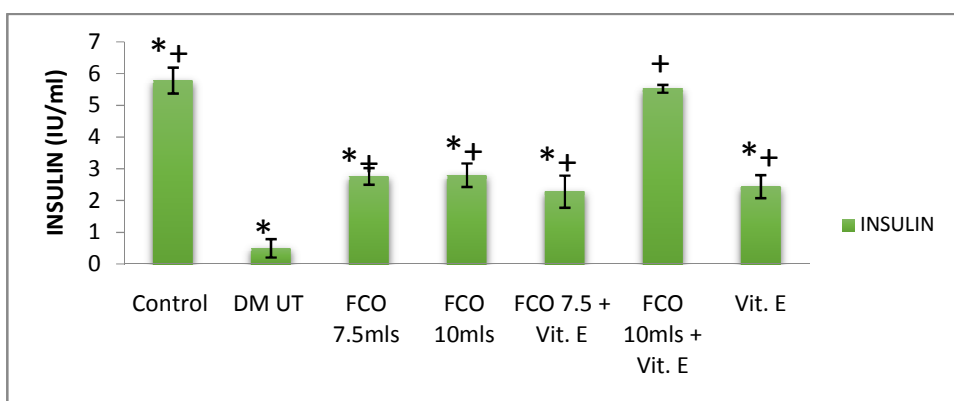


Chart 6. Insulin level change after 4 weeks of treatment (Unit 2 Experiment)
 Values are expressed as mean \pm S.E.M, n=5. Mean differences was compared using the one way analysis of Variance (ANOVA). Significant values were determined at $P \leq 0.05$ level and designated as (*) when compared with control and (+) when compared with diabetic untreated.

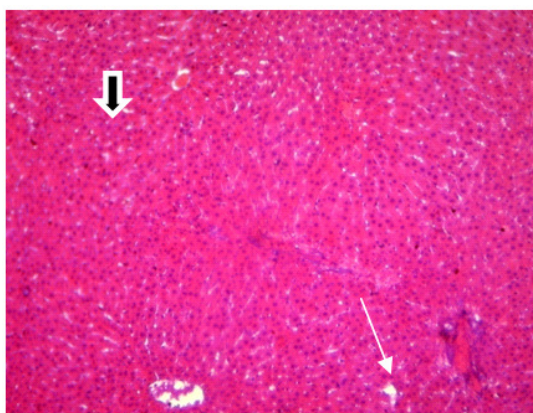


Plate 1. Photomicrograph of control group liver tissue in diabetes untreated group after 2 weeks x 100 magnification: Photomicrograph reveals histology of normal liver tissue. Thin arrow=Hepatocytes, Thick arrow=Sinusoids

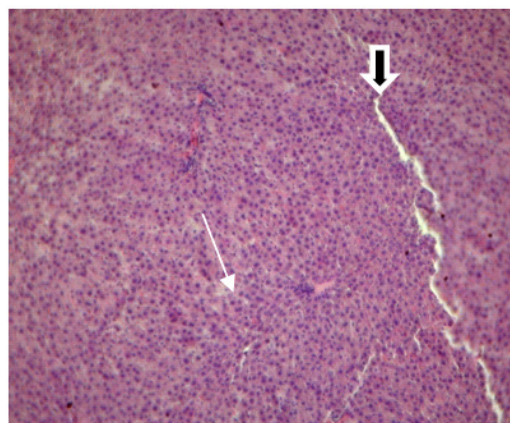


Plate 2. Photomicrograph of diabetes untreated group after 4 weeks (H and E), x100 magnification. Photomicrograph reveals diabetic liver tissue compared to the control. Thick arrow=Sinusoids, Thin arrow=Hepatocytes

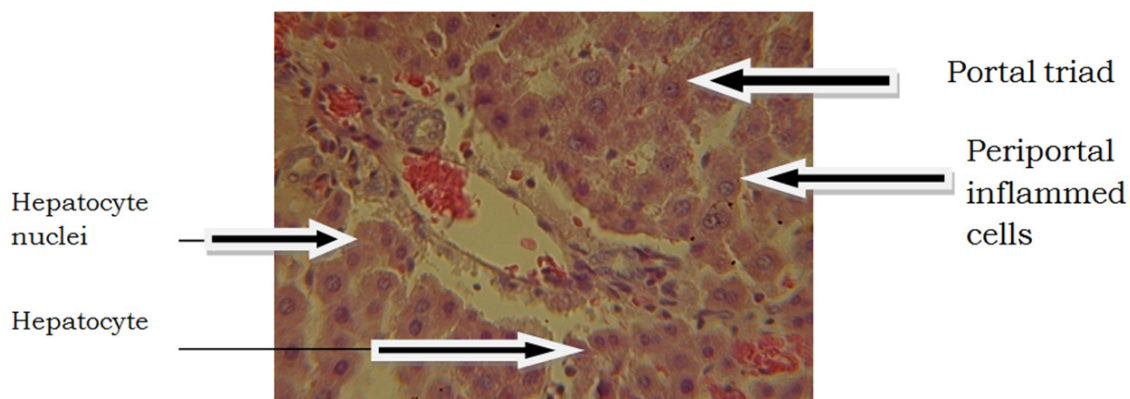


Plate 3. Photomicrograph of liver tissue treated with Vitamin E. 50mg/kg (H and E), x400 magnification. Photomicrograph reveals normal liver tissue with mild periportal inflammation

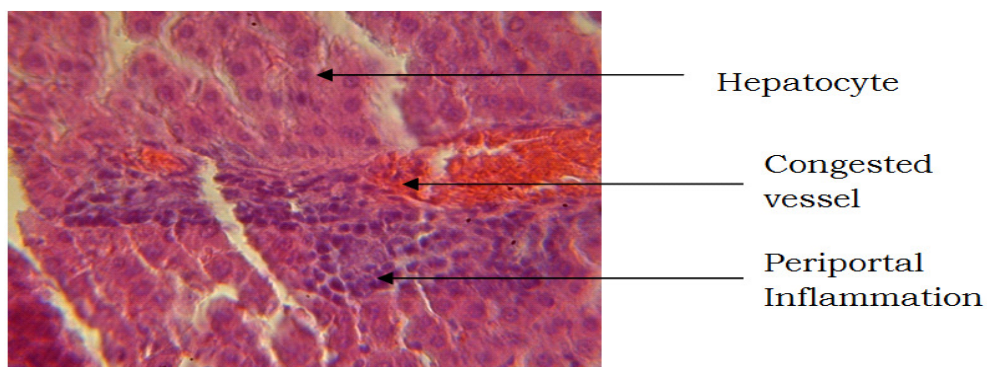


Plate 4. Photomicrograph of liver tissue treated with FCO 10 ml + Vitamin E (H and E) x400 magnification. Photomicrograph reveals normal liver tissue with marked periportal inflammation.

Fig. 1. Histological of Liver Tissue

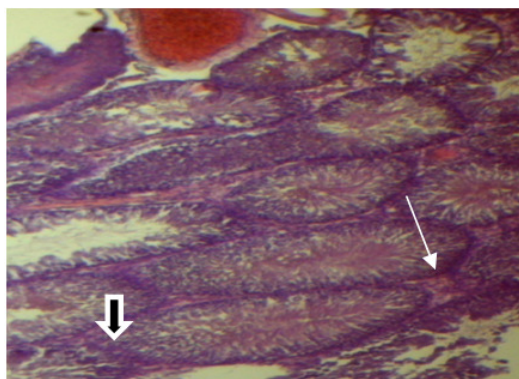


Plate 1. Photomicrograph of Control group of testes (H and E), ×10 magnification
Photomicrograph reveals normal testes Thin arrow=Lumen, Thick arrow= interstitial cells of laydig

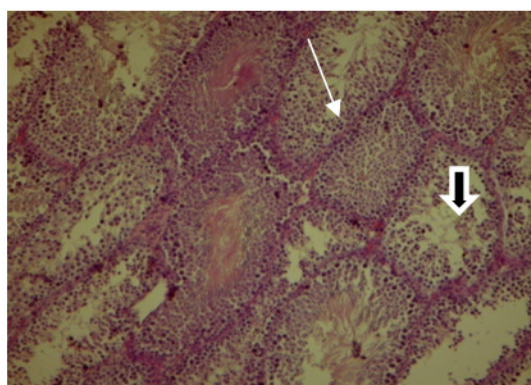


Plate 2. Photomicrograph of testicular tissue in diabetes untreated group (H and E), ×40 magnification: Photomicrograph reveals diabetic untreated testes as compared with the control. Thick arrow=interstitial cells of laydig, Thin arrow=Lumen

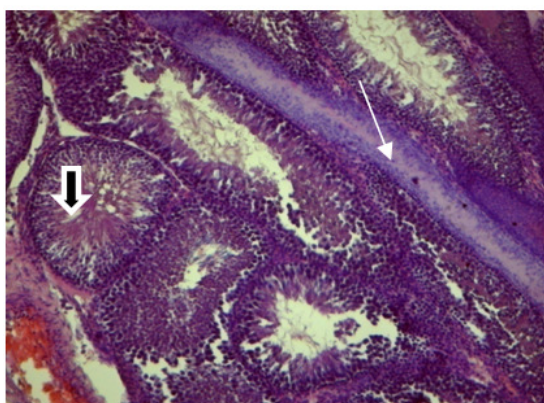


Plate 3. Photomicrograph of group (H and E), ×40 magnification: Photomicrograph reveals normal testes as compared with the control. Thin arrow=Lumen, Thick arrow=interstitial cells of laydig

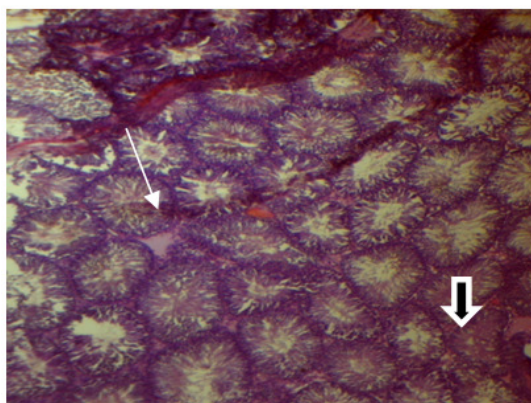


Plate 4. Photomicrograph of testicular tissue in 10ml + Vitamin E treated group (H and E), ×40 magnification: Photomicrograph reveals diabetic testes as compared with the control. Thick arrow=interstitial cells of laydig, Thin arrow=Lumen

Fig. 2. Histo-Architectural Outcome of Testicular tissues

4. DISCUSSION

Medicinal plants are commonly used in developing countries as alternatives 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. Below are an explanation and theoretical structure of the findings from this study.

Chart 1 shows the effect of FCO and Vitamin E on body weight of Wister rats after two weeks of treatment. The result shows a 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 a significant decrease in body weight within two weeks of treatment in alloxan-induced diabetes.

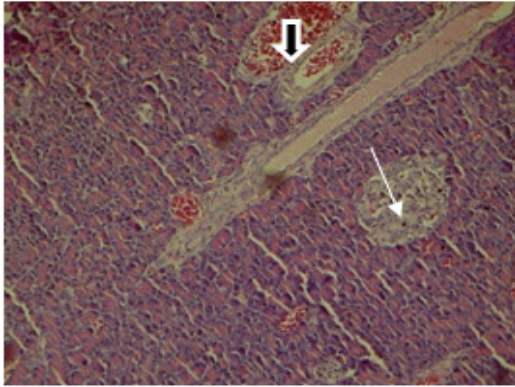


Plate 1. Photomicrograph of control pancreas(H and E), ×100 magnification
Result:Photomicrograph reveals normal islets cells surrounded by the exocrine cells.Thin arrow=Islet cells, Thick arrow=Blood vessels

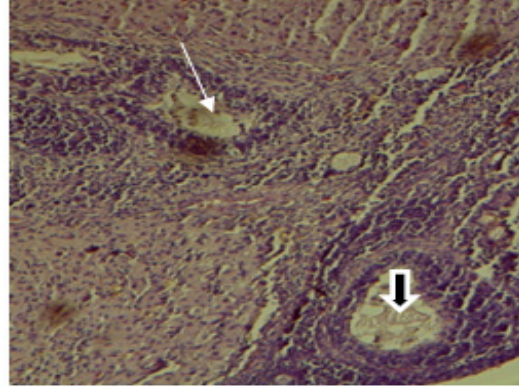


Plate 2. Photomicrograph of pancreas of diabetic untreated group (H and E), ×100 magnification. Photomicrograph reveals dense lymphocytic infiltrates around areas of islet cells. Thick arrow=Lymphatic infiltrate , Thin arrow=Islet cells

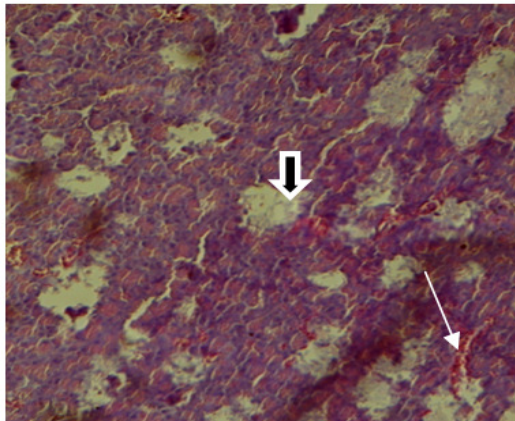


Plate 3. Photomicrograph of pancreas of diabetic untreated group (H and E),×100 magnification. Photomicrograph revealspancreatic islets cells looking shrunk anddepleted with a great reduction of islet cells. Thick arrow= Islet cells, Thin arrow= Capillary

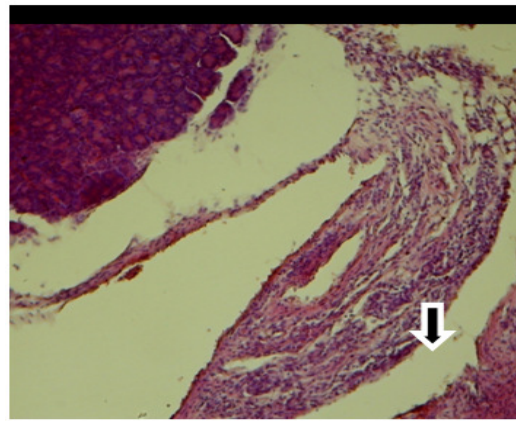


Plate 4. Photomicrograph of FCO 10ml+ Vitamin E (H and E), ×100 magnificationPhotomicrograph reveals very few isletcells with peripheral infiltration by mild tomoderate population infiltration by mild tomoderate population of lymphocytes. Also thereis perivascular lymphocytic infiltrate andfibrosis. Thick arrow= Lymphatic infiltrates

Fig. 3. Histological Analysis of Pancreatic Tissue Result

Chart 2 shows effect of FCO and Vitamin E on body weight (g) of Wister rats after four weeks of treatment. Compared with control, result shows a 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.

Charts 3 and 4 shows the effects of FCO and Vit. E on blood glucose levels of Sprague Dawley

Rats after two and four weeks of treatment respectively. A significantly elevated blood glucose level was seen in all experimental groups when compared with control. However, when experimental groups were compared with diabetes untreated group there was significant decrease in blood glucose level in all experimental groups except treatment with FCO alone. This implies that treatment with FCO and Vit E combined and separately improved blood glucose levels; while FCO separately (High

and Low doses) did not reduce blood glucose in alloxan induce diabetes. Chart 4 also shows a significantly elevated blood glucose level in all experimental groups as compared to control. However, when experimental groups were compared with diabetes untreated; there was significantly decreased blood glucose level in all experimental groups. This implies that treatment with FCO at all doses and Vit. E separately and combined significantly improves blood glucose level.

Charts 5 and 6 show the effect of FCO and Vit. E. on insulin levels of Sprague Dawley Rats after two and four weeks of treatment respectively. Results show significantly decreased insulin level in FCO 7.5mls and FCO 10 mls given separately while in combination with Vit. E groups were not significantly affected. However, when experimental groups were compared with diabetes untreated there was a significant increase in insulin level. This implies that treatment with FCO separately improves insulin level both combined and separate Vit. E groups showed additional improvement in alloxan-induced diabetes. For Chart 6, a significantly decreased insulin level was seen in all experimental groups except FCO, with high dose combined with Vit. E as compared with control group. In addition, when experimental groups were compared with diabetes untreated there is the significant elevation of insulin level in all experimental groups. This implies that treatment with FCO separately improves insulin level in all experimental groups; in addition, FCO in high dose combined Vit. E showed added improvement in diabetes.

In the present study, the antidiabetic effects of fresh coconut oil (FCO) were compared with the effect of the standard agent, Vitamin E. It was found out that blood glucose concentration was drastically reduced in diabetic rats treated with FCO. In addendum, serum insulin concentration was increased by the treatment with FCO in diabetic rats. The reduction of blood glucose in FCO treated diabetic rats may be attributed to the increased level of insulin as explained above [16]. Diabetic rats have been shown to have reduced of nitric oxide (NO) synthase activity, an enzyme required for the production of nitric oxide. Nitric oxide (NO) signifies as one of the signalling molecules participating in the modulation of the intracellular redox condition.

Previous studies reported that nitric oxide (NO) acts as a physiological modulator of hormones

secreted by islet cells. L-arginine, which is a precursor of NO can improve insulin secretion and then reduce hyperglycaemia, which subsequently provides beneficial actions, which are associated with increased NO formation in treated Type 2 diabetes patients [17]. There are reports that L-arginine and NO can prevent β -cell damage in alloxan-induced diabetic rats.

5. SIGNIFICANCE OF STUDY

Findings from this work will provide an understanding of the specific effects of dietary coconut consumption on diabetes mellitus. Hitherto, study has established that;

- i. FCO has the potential to combat diabetes mellitus
- ii. FCO can significantly ameliorate deteriorating liver tissues as evident in duration-dependent histo-architectural changes in hepatocytes following intake of FCO
- iii. FCO causes only ameliorative effects but does not offer any curative effect in diabetic rats. This implies that these FCO can serve as a good diet for a diabetic patient.
- iv. FCO restores cellular structure and functions of the Pancreas, Liver and Testes of diabetic rats.

6. CONCLUSION

Treatment of diabetic rats with FCO significantly improved morpho-functional outcomes in alloxan-induced, diabetic Sprague Dawley Rats. In this study, FCO treatment was seen to synergise 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 doses.

7. RECOMMENDATIONS

Due to its ameliorating effect on diabetic-induced tissues, we recommend the frequent intake of FCO for diabetics as it may help improve or resuscitate degenerating pancreatic beta cells, whilst improving diabetes mellitus.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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