Nutrient composition and ameliorative effects of Cocos nucifera products on Alloxan-induced diabetic wistar rats

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Abstract

Using standard methods, this study investigated the ameliorative effects of coconut products during alloxan-induced diabetic conditions. Experimental animals were divided into five groups, group 1 served as normal control rats fed only rat chow and saline, group 2 were diabetic control rats intraperitoneally treated with 150mg/kg body weight alloxan monohydrate, group 3 were diabetic rats orally treated with 4ml/day of coconut milk, group 4 were diabetic rats orally treated with 4ml/day of coconut water, and group 5 were diabetic rats orally treated with 4ml/day of a mixture of coconut milk and coconut water. The coconut products had high moisture, fats, potassium, magnesium, and sodium contents. Coconut milk exhibited the most effective glucose lowering effect, and on the 21st day. The total cholesterol was completely normalized on treatment with coconut milk after alloxan induced diabetes, while the administration of the mixture of coconut milk and water had a comparable effect to administering only coconut milk on HDL, LDL, and TG. The alloxan-induced derangements on SOD, catalase and GPx were completely normalized after the coconut milk administration, while the mixture of coconut milk and water restored only SOD and GPx, and coconut water, ineffective on most of the antioxidant enzymes. Coconut water was ineffective on the RBC and HB of diabetic rats, while coconut milk and the mixture of coconut milk and water showed the most hematoprotective effect. This study has shown the effectiveness of coconut products in the management of diabetes, with coconut milk the most effective.

Keywords: Diabetes; Antioxidant Enzymes; Coconut; Lipid Profile; Hematoprotective; Cholesterol.

1. Introduction

Diabetes mellitus is a multi-systemic complex disorder primarily caused by absolute or relative insulin deficiency that deprives the body the ability to effectively carry out glucose homeostasis (Aladodo et al. 2013, Ezeja et al. 2014). Diabetes mellitus may occur due to impairment of β-cells of the pancreas that thus decreases the secretion of insulin. It may also result from resistance to the functionality of insulin in circulation by insulin receptors (ADA, 2010). In most cases, diabetes mellitus manifests as aberration in lipids, proteins, carbohydrates, and blood parameters (Andreoli et al. 1990, Lebovitz, 1994). This eventually causes the accumulation of both fasting and postprandial glucose in the blood, resulting to various complications (Tierney et al. 2002, Rother, 2007). In event of persistent or recurrent hyperglycemia during diabetes, Sharma, (1993) reported the possible development of body proteins glycations leading to complications that affects various organs, nerves and arteries. Al-Khoury et al. (2006) have shown that patients suffering from diabetes mellitus show signs of serious derangement in several hematological parameters. The blood is an essential fluid comprised of the Red Blood Cells, White Blood Cells, and platelets, maintained in homeostatic concentrations in the serum. The blood serves many key roles such as nutritional, homeostatic and immunologic processes (Oze, 1992), tissue and pulmonary respiration, endocrine processes, and excretion of metabolic wastes (Adebayo et al. 2005). Ezenwaka et al. (2008) reported a high prevalence of anemia in patients with Type 2 diabetes, as much as 27% (Dipta et al. 2009). In addition, diabetes induces and promotes hematological disorders, where the occurrence of anemia in a hyperglycemic patient results from non enzymatic glycosylation or stiffening of the membrane proteins of the Red Blood Cells (Kennedy and Baynes, 1984). Further, the functionality of the pancreas is partly conditioned by the metabolism of active oxygen (Kaliavani et al. 2008). Researchers have reported the impairment of antioxidant enzyme system to occur as a pathological consequence of diabetes (Preetha et al. 2012). The generation of free radicals has been found as one of the characteristic effects of alloxan, a diabetes inducing agent, and free radicals contributes extensively to the generation of secondary complications in diabetes (Thomson and McNeil, 1993, Thormalley et al. 1996). In hyperglycemic conditions the production of reactive oxygen species leads to membrane damage and lipid peroxidation. The reduction of antioxidant defense system caused by increase in circulating levels of oxidants such as hydroxyl radical, superoxide radical, and hydrogen peroxide have been implicated in hyperglycemic conditions (Rahimi et al. 2005, Vincent et al. 2004). In hyperglycemic conditions, Baynes, (1991) and Pari et al. (2005) posited that in addition to the generation of free radicals during the pathogenesis of diabetes mellitus, oxidative stress can equally occur as a result of formation of peroxides, non-enzyme protein glycosylation, impairment of antioxidant enzymes, and auto-oxidation of glucose. This suggests that during diabetic conditions, alterations in the metabolism of macromolecules significantly elevates the generation of reactive oxygen species (Mohamed et al. 1999, Yue et al. 2003). Furthermore, the predominance of low density lipoproteins, triglyceride elevation, and reduction in levels of high density lipoproteins which are in all, consequent of plasma lipid and lipoprotein aberration, has been implicated in diabetic conditions (ADA, 2003). Thus, diabetic patients are largely sus-
ceptible to all clinical manifestation of atherosclerosis such as peripheral vascular diseases, cerebrovascular events, and coronary artery diseases (Mohamed et al. 1999, Cavalli et al. 2007, Torrico et al. 2007, Figueiredo and Modesto-Filho, 2008, Janebro et al. 2008). The effects of synthetic drugs in the management of diabe-
tes have been beneficial through the provision of good glycemic control. Unfortunately, despite the occurrence of numerous side
effects, none of those synthetic agents have completely controlled
terms and provisions of an extended access for diabetes. After the animals
 were also used to treat obesity problems, and for improved absorption and
together with Omotosho and Odeyemi (2012), there are also
ve, acceptable and more affordable (Valiathan, 1998). Herbal medicine which involves the utilization of plant parts like stems, roots, barks, leaves, and fruits (Akinyemi et al. 2005) in Africa, were the sole source of medical health care (Eseyin et al. 2005), and were also used to treat diseases all over the world (Aghbor et al. 2007). Coconut, an important member of the family has been utilized effectively in the treatment of a wide variety of health problems (Anosike and Obi-
doa, 2010). According to Omotosho and Odeyemi (2012), there are two types of nutritionally different liquids in coconut, the milk
water and coconut milk. Coconut milk is a white sweet liquid, extracted from a pulvzerized coconut meat. It possesses a refreshing test and attrac-
tive color, attributable to the high sugar and oil content (Omotosho and Adeyemi, 2012). Nair (2019) found coconut milk to contain a
complex mixture of nutritional constituents like minerals, vita-
mains, and carbohydrates. Osazuwa and Ahonkhai, (1990) and Adodo, (2002) reported that coconut water is used in an intravenous fluid to dispel complications arising from drug overdose, and poi-
sion. It is also used as a source of immune system boost and quick energy, metabolic rate enhancer, preventive therapy for over-
weight and obesity problems, and for improved absorption and
digestion of amino acids, minerals, and vitamins (Awad, 1981).
Coconut has also found application in the management of diabetes
due to its role in the improvement of insulin secretion and en-
hancement of blood glucose utilization. Notwithstanding the folk-
lores on the nutritional and health benefits of coconut products, there is paucity of experimental data on the specific biochemical effect of coconut milk and water during hyperglycemic conditions. It is on this forgoing, that this study was carried out to investigate the nutrient compositions of coconut milk and water, and their effects in hyperglycemic conditions.

2. Materials and methods

2.1. Sample collection and preparation of Cocos nucl-
era products

Thirty matured coconuts were purchased from Choba Market in Rivers State Nigeria. The coconut fruit was dehusked using a
sharp knife, and afterwards, the shell was broken, then the water
was collected from the “eyes” area. Exactly 100g of coconut meat
was sliced and put in an electric blender. A stock solution of co-
conut milk was obtained by blending the 100g of sliced coconut in
200ml of distilled water and filtered using a sieve. The filtrate
was boiled with constant stirring for another 60 mins to produce
the crude milk which was allowed to cool to room temperature.
The extracts were preserved by refrigeration at 4°C, but the cocon-
ut water was discarded each week and replaced with freshly pre-
pared ones.

2.2. Proximate composition and phytochemical screen-
ing

Proximate analysis of the samples for carbohydrate, crude fat, ash, crude protein, fiber and moisture contents were carried out accord-
ing to standard methods of AOAC, (1990).

2.3. Mineral content determination

The minerals were determined by atomic absorption spectropho-
tometry. Fifty (50) ml of the sample was evaporated to dryness using a
muffle furnace until a residue of constant weight was obtained. Exact 20.0 ml of 2.5% HCl, was added to the residue to extract
the minerals, and then heated in a steam bath to reduce the volume
to about 7.0ml, before being transferred quantitatively to a 50ml
volumetric flask. The volume was made up to 50ml with deionised
water and transferred into clean polyethylene bottles, and the min-
eral contents were determined using an atomic absorption spectro-
photometer (Buck Scientific model 210 VGP) and flame photome-
ter (Jenway model).

2.4. Experimental animals

Sexually mature male Wistar rats of body weight 154g±2 were
obtained from the animal house of the Department of Biochemis-
try, University of Port Harcourt Choba Rivers State. The animals
were housed in a cage at the Animal House and fed a standard diet (Top feed grower’s mash) and water ad libitum. The rats used in
the present study were maintained in accordance with guidelines
of the internationally accepted principle for laboratory animal use
and care (NIHES, 1985).

2.5. Induction of diabetes

The rats were injected with 150 mg/kg body weight single intra-
peritoneal dose of alloxan monohydrate dissolved in sterile normal
saline. Seven days after alloxan injection, rats with marked hyper-
glycemia (fasting blood glucose ≥ 200 mg/dl) were separated and
divided into five groups:

Group 1: Control rats only fed on normal diet and normal saline.
Group 2: Diabetic control
Group 3: Diabetic rats orally administered 4ml kg⁻¹ body weight
cocnut milk daily using an intragastric tube.
Group 4: Diabetic rats orally administered 4ml kg⁻¹ body weight
cocnut water daily using an intragastric tube.
Group 5: Diabetic rats orally administered 4ml kg⁻¹ body weight
cocnut milk and water (in a ratio of 1:1) daily using an intra-

2.6. Biochemical analysis

The animals were sacrificed at the 21st day by anaesthesizing with
10% chlororm phosphate. Blood was collected and the peritoneum
was cut open, and the pancreas quickly harvested. The pancreatic
tissues were placed in 10% formalin solution, and immediately
processed by the paraffin technique. Sections of 5μm thickness
were cut and stained using haematoxylin and Eosin (H & E) for
histological examination. The photomicrographs of histological
sections were obtained. The collected blood was centrifuged at
3000 rpm for 10mins to separate sera. Blood glucose was meas-
ured using the glucose oxidase method

2.7. Determination of body weight and organ weights

Body weight of the entire animal in each group was noted on the
before treatment, at 48hrs, 7, 14, and 21 days of the experiment
period. The weight difference was calculated. After the animals
were sacrificed, the pancreas, liver, heart, kidneys and spleen were
isolated, washed with saline and weighed by using an electronic
balance.

2.8. Determination of hematological parmers

The packed cell volumes (PCV), White blood cell (WBC) counts,
Red blood cell (RBC) counts, Hemoglobin (Hb) Concentrations,
and Platelets counts, were obtained using an Automated Hematol-
omy Analyzer–MC-2800 (Mindray Company, China).
2.9. Lipid profiling

Plasma total cholesterol, triglycerides, and HDL were determined enzymatically using commercially available kits (Randox kits). From the results, LDL cholesterol, using the formular of Friedewald et al. (1972).

2.10. Determination of antioxidant enzymes

2.11. Estimation of superoxide dismutase (SOD) activity (McCord and fridovich (1969))

Sample extract (20ml) and 2.5 ml of 0.05 M carbonate buffer (pH 10.2) were mixed together and equilibrated in the spectrophotometer. In addition, 0.3 ml of 0.3 mM freshly prepared adrenaline was added and mixed by inversion. The increase in absorbance at 480 nm was monitored spectrophotometrically at 30 seconds intervals for 3mins.

2.12. Determination of catalase activity (aebi, 1984)

Distilled water (2. 5ml) was pipetted into test tube containing 0.5 ml H2O2; and about 40µl sample was added and mixed thoroughly. Rate of decomposition of hydrogen peroxide was read at 240nm at 30sec interval for 5 mins.

2.13. Determination of malondialdehyde (ohkawa et al. 1979)

Normal saline (0.5ml) was pipetted into a test tube containing 0.5ml of the serum sample. About 2ml of thiobarbituric acid (TBA)/trichloroacetic acid (TCA) mixture was added, allowed to boil for 1 hour, cooled to room temperature, and centrifuged at 4000rpm for 5min. The clear supernatant was read at 532nm.


To a test tube containing 0.5ml of the sample was added 0.5ml (50%) of TCA and the solution was mixed and centrifuged at 2.0 x 10³rpm. Then, 1ml of the supernatant was mixed with 2ml of 0.01m DTNB reagent (Ellman’s reagent) and kept away from direct light for 15 to 20 minutes. The absorbance at 412nm was recorded. Then, standard glutathione was added to a mixture of 1.5ml phosphate buffer and 2ml of DTBN, and absorbance was read at 412nm after 15 minutes. The concentrations of glutathione (µg/ml) were traced from the standard curve for glutathione.

2.15. Determination of glutathione peroxidase (GPX) (Paglia and valentine, 1967)

Glutathione peroxidase (GSH-px) activity in the sample was measured using Randox GSH-px kit according to the method of Paglia and Valentine, (1967).

2.16. Statistical analysis

All data were subjected to statistical analysis. Values are reported as Mean ± Standard error of mean (SEM) while one way ANOVA was used to test for differences between treatment groups using Statistical Package for Social Sciences (SPSS) version 20. The results were considered significant at p-values of less than 0.05 (p<0.05).

3. Results

| Table 1: Proximate Composition of Cocos Nucifera Products |
|---------------------------------|--------------------|--------------------|
| **Composition** | **Coconut milk** | **Coconut water** | **Coconut milk+water** |
| **PROTEIN (%)** | 3.77±0.15a | 0.80±0.07b | 2.94±0.28a |
| **CARBOHYDRATE (%)** | 2.93±0.27a | 3.77±0.54a | 3.33±0.40a |
| **LIPID (%)** | 11.98±0.047a | 0.033±0.005b | 5.24±0.73a |
| **MOISTURE (%)** | 80.78±0.35a | 94.90±0.45a | 87.98±0.15a |
| **ASH (%)** | 0.49±0.05a | 0.46±0.05a | 0.47±0.02a |
| **FIBRE (%)** | 0.03±0.02b | 0.01±0.01a | 0.02±0.01a |

Values represent means ± standard deviations of triplicate determinations. Values with similar superscript letter (a-c) across the column denotes no significant difference at p<0.05.

The proximate content of coconut milk, water and a mixture of both extracts are presented in Table 1. The results show that coconut milk possesses more protein and lipid content, while coconut water contained the highest moisture content among the three products, and hence more susceptible to microbial infestation. No significant change was recorded for the carbohydrate, ash, and fibre content among the three compared coconut products. The protein content of coconut milk was comparable to the protein content of Tiger nut milk but however higher than the lipid content as shown by Awoniran and Udeozor, (2014). Also, the protein content of coconut water was higher than the protein content of fresh watermelon (Fila et al. 2013). The moisture content of coconut milk reported in this study (80.78%±0.35) was comparable to the moisture content of groundnut milk (Adieyi et al. 2013) and Tiger nut milk (Awoniran and Udeozor, 2014). The lipid content of both coconut milk and the mixture of coconut milk and water was higher than the values reported for cow milk (Adieyi et al. 2013), while the fibre content of the Tiger nut milk as reported by Awoniran and Udeozor was higher than the fibre content of coconut milk reported in this present study.

<table>
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<tr>
<th>Table 2: Mineral Contents (Mg/100 ml) of Cocos Nucifera Products</th>
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<tr>
<td><strong>Minerals</strong></td>
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<td><strong>Potassium (K)</strong></td>
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<td><strong>Calcium (Ca)</strong></td>
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<td><strong>Phosphorus (P)</strong></td>
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<td><strong>Manganese (Mn)</strong></td>
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Values represent means ± standard deviations of triplicate determinations. Values with similar superscript letter (a-c) across the column denotes no significant difference at p<0.05.

Table 2 shows the mineral contents of each of the coconut products. In a decreasing order, the mineral composition was found as follows K>P>Na>Ca>Mg>Fe>Mn>Zn>Cu, while in the coconut products, the levels of mineral composition was in this order, coconut milk>coconut milk + water >coconut water. Coconut water had the highest composition of potassium and sodium but was least in composition of other mineral constituents. The phosphorus, calcium, and zinc contents of all the coconut products evaluated were greater than those for popularly consumed fruit juices, strawberry, raspberry, blueberry, and gooseberry (Marjanovic-Balaban et al. 2012). The potassium and magnesium content of the mixture of coconut milk and water was greater than the values reported for the juice from Psidium guajava, Anona muricata, Citrus lanatus, and Citrus sinensis, while the potassium content of coconut milk found in this study were lower than the potassium content of Carica papaya juice (Ekpete et al. 2013) but comparable with apple and pineapple juice (Ekpete et al. 2013). The manganese and sodium content (2.00mg/100ml±0.34 and 48.26mg/100ml±5.73) of coconut milk presented in Table 2, was higher than the contents of Morinda citifolia juice and placebo.
juice used as sports drink (Anugweje, 2004) while the iron content of the coconut products was found lower than those reported for the sports juice (Anugweje, 2014).

The results of the effect of administration of coconut products on body weight, relative to the treatment duration was presented in Fig.1, while the effects on organ weight after 21 days administration was shown in Fig. 2. The results showed no significant effects of the extracts after 48hrs and 7days treatments but positively modulated the diabetogenic effects of alloxan administration on body weight on the 14th and 21st day (Fig. 1). Administration of coconut water proved more deleterious on organ weight at the 7th day of intake, as a significant decrease in body weight was evident after the oral treatment with coconut water. The body weight of the experimental animals continuously depreciated with increasing treatment periods on alloxan, however, at the 21st day, the ameliorative effect of the coconut products were optimal, with coconut milk shown to be the most effective. In Fig. 2, the administration of both coconut milk and the mixture of coconut milk and water were recorded to be the most hepatoprotective as well as the most with positive modulation on the kidney. The effect of the diabetes inducing agent on the kidney weight remained unaffected by coconut water while inducing agent on the kidney remained unaffected by coconut water. The derangement of the spleen remained unchanged after administration of the coconut products.

On the blood glucose levels of alloxan induced diabetic rats orally treated with coconut milk, water, and a mixture of both coconut milk and coconut water (Fig 3), the results showed that none of the coconut products used had a complete restorative effect. The mixture of coconut milk and water had as much ameliorative effect on blood glucose level as the coconut milk only on the 14th day. However, this comparable restorative effect depreciated between the 14th and 21st days of treatment. A similarly comparable effect of these extracts used for this study on blood glucose was reported for a standard antidiabetic agent, glibenclamide, and M. malabraticum leaf (Balamurugan et al. 2014). In addition, on the 14th day, the effectiveness of coconut milk for the management of diabetes mellitus was in similar pattern to the reports of Shamon et al. (1994) for Costus speciosus rhizome extract on alloxan induced diabetic albino rats. In this study, the near restorative effect of coconut milk on the blood glucose levels may imply that coconut milk induced the reversal of insulin resistance or increased the secretion of insulin by possibly regenerating the damaged pancreatic β-cells in the diabetic rats (Sezik et al. 2005).

The lipid profile of the diabetic rats treated with coconut products were shown in Fig. 4. According to Adeyemi et al. (2009), diabetes mellitus propagates profound aberrations in serum lipid profile and lipoprotein levels, thus increasing the susceptibility to coronary heart diseases. After 21 days oral treatment in this study, alloxan significantly increased the LDL, TC and TG levels of experimental animals in group 2, which consequently reduced on administration of coconut products. In diabetic conditions, al-Shamony et al. (1994) posited that the most commonly obtained lipid dysfunctions are hypertriglyceridemia and hypercholesterolemia. In line with this, the total cholesterol and triglyceride levels were the most significantly deranged after treatment with the diabetogenic agent (Fig. 4). The administration of coconut milk proved most effective, but comparable in all cases with the effect of treatments using the mixture of coconut milk and water. Only the total cholesterol levels of diabetic rats treated with coconut water, was completely restored after 21 days. The decreased HDL level observed during diabetic condition was incompletely ameliorated by the coconut products, with coconut water, the least effec-
tive. From the findings of Devi et al. (2012), Echinochloa crusgalli showed more hypolipidemic potentials, when compared to the results for the coconut products in this study. Whole plant extract of Sarcostemma secanone and rhizomes of turmeric were also reported to possess hypolipidemic potentials (Mohan et al. 2013, Jeevangi et al. 2013) similar to the cholesterol lowering effect of coconut milk reported in this study.

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**Fig. 3:** Effect of Cocos Nucifera Products on Blood Glucose Levels of Alloxan Induced Diabetic Rats.

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**Fig. 4:** Lipid Profile of Diabetic Rats Orally Treated with Cocos Nucifera Products.

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**Fig. 5.1:** SOD Levels of Diabetic Rats Treated with Cocos Nucifera Products.

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**Fig. 5.2:** Catalase Levels of Diabetic Rats Treated with Cocos Nucifera Products.
catalase activity (Fig. 5.2) except for the incomplete restoration by the mixture of coconut milk and water. Intraperitoneal treatment with alloxan significantly increased the MDA levels from 8.93mMol/L ± 0.42 to 19.93mMol/L ± 0.55 (Fig. 5.3). The increase in MDA levels could be suggestive of depletion of antioxidant enzyme defense system (Mori et al. 2003). The results also showed that the most effective coconut product for reducing the levels of MDA levels was the coconut milk. However, other coconut products significantly reduced the MDA levels compared to the levels during diabetes. The administration of the coconut products proved ineffective in increasing the activity of GSH after depletion by alloxan (Fig. 5.4). Similar to the findings of this study on GSH activities, the result was in agreement with the findings of Iranloye et al. (2013) after the administration of 7.5ml of coconut oil, however, a consequent increase in the activity of GSH was observed on increment to 10ml oral treatment. As shown in Fig. 5.5, the activity of GPx was normalized in the diabetic rats after the oral treatment with coconut milk and the mixture of coconut milk and water. This could imply that coconut milk possesses strong antioxidant potentials. It is possible that administration of coconut milk enhances response to oxidative cells by enhancing the secretion of insulin.
RBC, HB, and platelets were completely reversed by treatment with Cocos nucifera milk. This resulting increase in HB and RBC count on administration of Cocos nucifera milk might have resulted from its peroxide lowering effect leading to the decreased hemolysis of RBC (Crouch et al. 1981). Also, oral treatment with the mixture of Cocos nucifera milk and water completely reversed the levels of RBC, PCV, HB, and platelets. Coconut water had no effect on RBC and HB of diabetic rats as shown in Fig 6.3 and 6.4 respectively, but induced a significant increase in PCV (Fig. 6.1) and WBC (Fig. 6.2) while it had a comparable effect on the platelets with other coconut products evaluated in this study (Fig. 6.5). None of the coconut products normalized the levels of WBC, but however significantly lowered the levels in comparison to the effect obtained in diabetic conditions (Fig. 6.2).

The results of the effects of coconut products on hematological indices of alloxan induced diabetic rats were shown in Figs. 6.1-6.5. Halim and Ali (1996) reported that decreased hematological parameters are testament to anemic conditions. The intraperitoneal administration of alloxan in this study, showed a hyperglycemia-induced anemia. Sheela and Augusti (1992) remarked that in hyperglycemic conditions, the decrease in total hemoglobin levels results from the reaction of the consequent excess blood glucose and hemoglobin, thus forming glycated hemoglobin. The results of this study showed that these derangements in the levels of PCV,
The results of the histopathological examination of the pancreatic cells were shown in Plate A-D. The result shows a near complete loss of the pancreatic islet on administration of alloxan (Plate B), which were regenerated mostly by the oral treatment of coconut milk (Plate C), while treatment with coconut water had minor regenerative effect (Plate D).

4. Conclusion

Our findings showed that coconut milk contained the best combination of nutrients among the other coconut products evaluated in this study. All the coconut products significantly improved the blood glucose levels after alloxan-induced alterations, but showed significant hypolipidemic, antioxidant, and hematoamellar regenerative effect (Plate D).

5. Conflict of interest

The authors declare no conflict of interest regarding the publication of this article.

References
