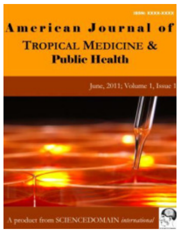

American Journal of TROPICAL MEDICINE & Public Health
1(3): 137-146, 2011

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Regenerative Effects of Coconut Water and Coconut Milk on the Pancreatic β–Cells and Cyto Architecture in Alloxan Induced Diabetic Wistar Albino Rats

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Research Article

Received 19th May 2011
Accepted 14th June 2011
Online Ready 8th November 2011

ABSTRACT

The effects of Coconut water and Coconut milk on the total body weight, blood glucose level and the cyto- architecture of the pancreatic tissue were studied. A total of 30 albino wistar rats were randomly divided into five experimental groups (n=6). Group 1 –normal control was fed on normal rat chow, Group 2,3,4,5 were made diabetic by single intraperitoneal dose of 100mg/kg b.wt. of alloxan monohydrate. Group 2- diabetic, non treated, were fed on normal rat chow, Group 3, and 4 were fed on coconut water ad libitum for 14 and 28 days respectively, and Group 5 were fed on coconut milk ad libitum for 28 days. The result shows a statistically significant decrease in total body weight in Group 2, 3 and 4 and a non statistically significant increase in body weight in groups 1 and 5. Also there was a statistically significant decrease in blood glucose level in the group fed with coconut water. The pancreatic histology also shows a regenerative effect with administration of both coconut water and especially coconut milk on the degenerative changes caused by diabetes. This study has shown that coconut water has a hypoglycaemic effects and coconut milk has a regenerative effect on the pancreatic cells of alloxan induced diabetic rats. The findings in this research may be of benefit in the management of diabetic patients.

Keywords: Coconut water; coconut milk; pancreatic cells; diabetes mellitus; pancreatic cyto-architecture.

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1. INTRODUCTION

Diabetes mellitus is a major degenerative disease in the world today (Ogbonnia et al., 2008). It is a serious lifelong condition that affects an estimated population of about 151 million (Zimmet et al., 2001) and a third of these goes about undiagnosed until many years after the onset (Betheside, 1995). Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia (Scoppola et al., 2001; Ugochukwu et al., 2003), lipoprotein abnormalities (Scoppola et al., 2001), raised basal metabolic rate (Avesani et al., 2001; Nawata et al., 2004; Owu et al., 2006), defect in reactive oxygen species scavenging enzymes (Kesavulu et al., 2000) and altered intermediary metabolism of major food substances (Avesani et al., 2001; Unwin et al., 2001; Nawata et al., 2004). Uncontrolled diabetes mellitus causes varied histopathological changes in different organs (Harold, 1978; Thomas, 1999).

Coconut water contains sugars, vitamins, minerals, proteins, free amino acids and growth promoting factors (Muanya, 2009). Coconut water should not be confused with coconut milk, although some studies have used the two terms interchangeably. The aqueous part of the coconut endosperm is termed coconut water, whereas coconut milk, also known as “santan” in Malaysia and Indonesia, and “gata” in the Philippines, refers to the liquid products obtained by grating the solid endosperm, with or without addition of water (APCC, 1994). Coconut water is served directly as a beverage to quench thirst, while coconut milk is usually used as a food ingredient in various traditional cooking recipes. The main components of coconut milk are water, fat and protein (Seow and Gwee, 1997), whereas coconut water contains mainly water. Unlike coconut water, coconut milk, which is the source of coconut oil, is generally not used in plant tissue culture medium formulations (George and Sherrington, 1984). Zakaria et al., (2006) showed that the aqueous extract of coconut meat exhibited anti-inflammatory and wound healing properties when tested on mice. Coconut milk is also essentially composed of high amount of protein, amino acids (glutamine, Arginine, lysine, leucine, proline), water, sugars (lactose), fats, vitamins (Ascorbic acid, Nicotinic acid, Biotin pantothenic acid), minerals (nitrogen, calcium, iron, phosphorus) (Anzaldo et al., 1980; Davis, 1982; Pehowich et al., 2000; Effiong, 2003).

Plants which have been shown to have hypoglycemic action, act on blood glucose through different mechanisms. Some of them may inhibit endogenous glucose production (Eddouks et al., 2003) or interfere with gastrointestinal glucose absorption (Musabayane et al. 2006); some may have insulin - like substances (Collier et al., 1987; Gray and Flatt, 1999); some may inhibit insulinase activity and some may increase secretion of insulin from the β cells of the pancreas i.e. pancreatotrophic action (Khan et al.,1990; Trivedi et al., 2004; Yadav et al., 2008), while others may increase beta cells in pancreas by activating regeneration of these cells (Shanmugasundaram et al., 1990; Jelodar et al., 2007).

2. MATERIALS AND METHODS

2.1 Animal Handling

Thirty (30) adult Wister rats weighing between 170g to 200g of both sexes were used. The animals were purchased from the Animal center of the Faculty of Basic Medical Science, Ambrose Alli University, Ekpoma and were kept in the animal laboratory of the Faculty of Basic Medical Sciences, Delta State University for the duration of experiment. They were fed with standard rat feed, and water *ad libitum*, but starved for 12 hr prior to commencement of experiment. All animal experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals (Pub. No. 85-23, Revised 1985).

2.2 Preparation of Coconut Milk and Coconut Water

A matured coconut fruit was obtained from Ubulu-uku market, Ubulu-uku in Delta State, Nigeria, and used for the study. The fruit was shelled, the nut removed and the water collected. The nut was then grated using a stainless grater. A stock solution of coconut milk was obtained by dissolving 50g of the grated mass of coconut in 500ml of distilled water, heated to 65^0c and the residues removed using a sieve. The solution was boiled for 30 minutes, and oil scooped. The solution was again boiled with constant stirring for another 30 minutes, and the crude milk was allowed to cool to room temperature. 50mg/kg body weight of coconut water and 50mg/kg body weight of coconut milk obtained were administered to the animals in 4.0ml of the vehicle via gastric incubation.

2.3 Experimental Groups and Treatment

The rats were randomly divided into five groups (n=6) and each rat was weighed before and after the experiment using an electronic weighing scale. Group 1 was the control group treated with distilled water; group 2, diabetic rat, non-treated, received water and rat chow, group 3 was diabetic, treated with coconut water of 14 days; group 4, diabetic, was treated with coconut water for 28 days; and group 5, diabetic were treated with coconut milk for 28 days.

2.3.1 Induction of diabetes

Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared solution of alloxan-monohydrate (100 mg/kg) (Pari and Mahesulari, 1999). The rats' fasting blood glucose (FBG) levels were estimated 3, 5, 7 days post treatment using One Touch® glucometer (Lifescan, Johnson & Johnson, California). Seven days later rats with blood glucose concentration above 200 mg/dL were considered diabetic and used for the study.

2.4 Histopathological Studies

The pancreas was removed, weighed and observed macroscopically. This organ was fixed in 10% formal saline for at least 48 hours, processed routinely and embedded in paraffin wax. Histological sections were cut at 5-6 µm and stained with routine haematoxylin and eosin (HE) and examined.

2.4.1 Statistical analysis

Results were expressed as mean ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA). Students t'-test at 95% level of significance was used to assess significant difference between the control and treated group.

3. RESULTS AND DISCUSSION

The results from this study shows that Groups 2 (139± 2.89)g, 3 (139± 2.06)g and 4(143 ±2.16)g had a statistically significant decrease ($P < 0.05$) when compared with the control (195± 2.01)g group in their final body weight (Table 1). This finding is consistent with previous reports of weight loss in diabetic rats as a result of increased catabolism (Arrais and Dib, 2006). The finding also suggests that coconut water play a role in the decrease in the treated rats' weight as previously reported that coconut water promotes weight loss (Nandakumaran et al., 2009). It is also worthy of note that there was an increase in weight in group 5 (184 ± 3.70)g fed with coconut milk though not statistically significant.

Table 1: Changes in body weight of Rats (g)

Groups	1	2	3	4	5
Initial Weight(g)	173 ±2.18	171 ±2.49	180 ±8.27	176 ±2.08	170 ±2.89
Final Weight(g)	195±2.01	139±2.89*	139 ±2.06*	134±2.16*	184 ±3.70
Weight difference(g)	20±0.17	-32±-0.37	-41 ±6.21	-42 ±-0.08	14±0.81

*Values are presented as means ± SD; *P < 0.05 compared with group I; Group abbreviations as in table.*

Also there is a statistically significant (p<0.05) decrease in blood glucose level (hypoglycaemic effect) of coconut water in group 3(98±6.12)mg/dl and 4(104±1.22.)mg/dl (Table 2). This is consistent with the finding of Salihu et al. (2009) who reported that coconut water extract from *C. nocifera* has a significant hypoglycemic effects in alloxan-induced diabetes. Campbell et al. (2000) also supports this fact when he stated some benefits of coconut water which improves insulin secretion and utilization of blood glucose.

Table 2: Changes in Glucose level of Rats

Groups	1	2	3	4	5
Final blood glucose level (mg/dl)	89±2.11	97.5±2.04	98.8±6.12	104.5±1.22	98.5±3.67
Initial blood glucose level (mg/dl)	83±2.41	244.7±4.10*	227±11.43*	217±11.40*	259±3.10*
Difference in blood glucose level (mg/dl)	6 ±2.41	147±2.45	128.2 ±4.11	121.5±4.09	160.5±2.00

*Values are presented as means ± SD; *P < 0.05 compared with group I; $^{+}$P < 0.05 compared with group 2; Group abbreviations as in table.*

3.2 Microscopic Examination of the Pancreas

The histology of the pancreas of the non diabetic control (Plate 1) was used to compare with the diabetic untreated group (Plate 2) diabetic coconut water treated groups (Plate 3 & 4) and coconut milk treated group (Plate 5) in other to find the effects of coconut water and coconut milk. In this study, the pancreatic ß cells were destroyed to induce diabetes with the help of alloxan (Prince and Menon, 2000; Jelodar et al., 2007). The non diabetic group showed multiple islets of Langerhans, blood vessels, intralobular ducts and a well defined septum, and pancreatic cells with normal exocrine and endocrine parts. The coconut water treated diabetic group showed sparsely dilated blood vessels, not clearly defined islets of langerhans, but milk treated diabetic group showed a clearly defined islets of langerhans, with multiple intralobular and serous acini. The exocrine and endocrine portions of the pancreas can be clearly visualized indicating improvement which shows a regenerative effect of coconut milk on the pancreatic histology.

Plate 1 (Control group, non treated, non diabetic pancreas of adult wistar rat) shows a well defined septum, multiple intralobular ducts. Multiple islets of Langerhans and blood vessels can be seen. Normal pancreatic tissue with endocrine and exocrine parts can be seen.

Plate 2 (Group 2 – non-treated diabetic pancreas of Wistar rats) shows disorientation of the serous acini at the exocrine portion. The ducts are undifferentiated with sparsely distributed blood vessels. The Islets of Langerhans cannot be clearly defined. The cyto-architecture is distorted.

Plate 3 (Group 3 – Coconut water treated diabetic pancreas of Wistar rats for 14 days) shows disorientation of the serous acini at the exocrine portion. The ducts are differentiated with sparsely distributed blood vessels. The Islets of Langerhans cannot be clearly defined. The septum is defined.

Plate 4 (Group 4 – Coconut water treated diabetic pancreas of adult wistar rats for 28 days) shows serous acini at the exocrine portion. The ducts are undifferentiated and the islets of Langerhans can be seen but not clearly defined**.**

Plate 5 (Group 5 – Coconut Milk treated diabetic pancreas of Wistar rats for 28 days) shows multiple intralobular ducts and serous acini. Multiple islets of Langerhans can also be seen. The exocrine and endocrine portions of the pancreas can be clearly visualized.

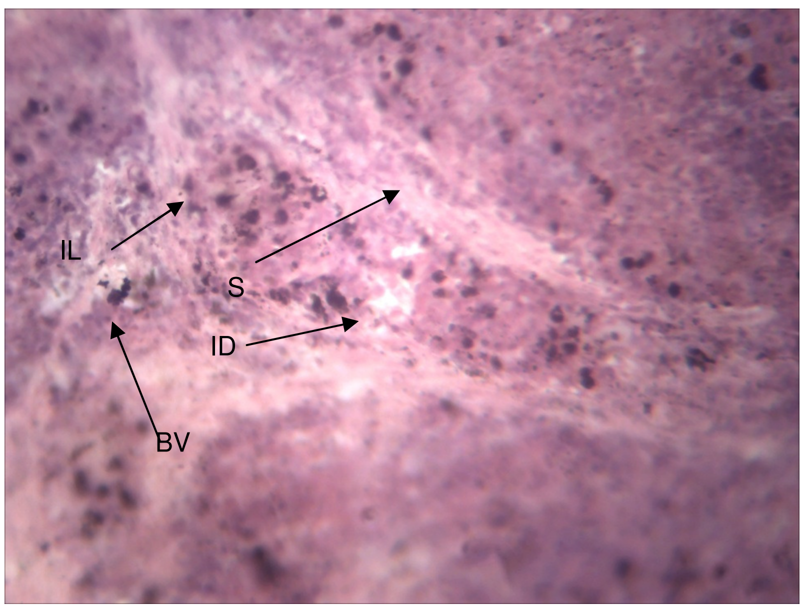

Plate 1: Coronal section of pancreas.
Group 1 (non-Diabetic pancreas of wistar rats for 28 days)
(H & E Stain, x100): Cross section of pancreas: S – Septum,
IL – Islet of Langerhans, ID – Intralobular Duct, BV – Blood Vessel

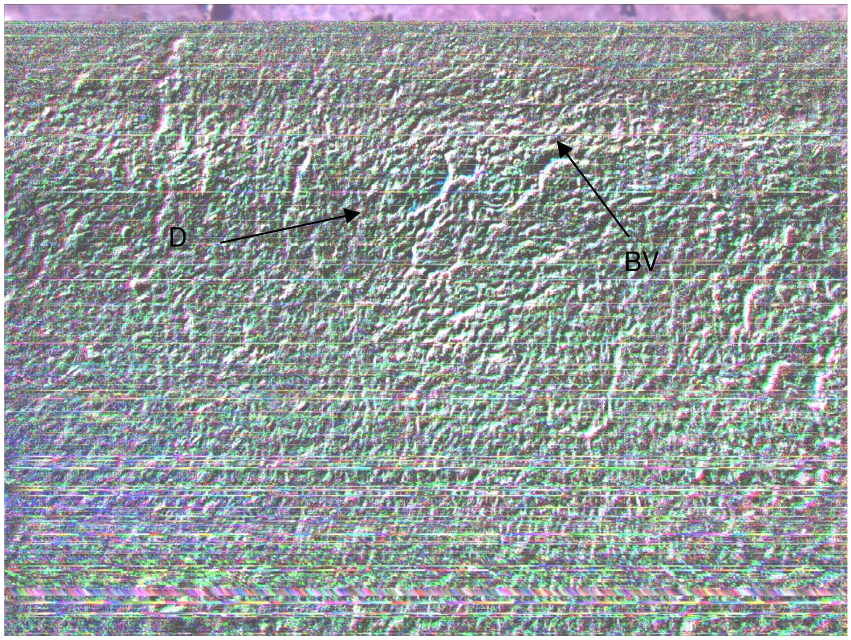

Plate 2: Coronal section of Pancreas.
Group 2 (non-treated diabetic pancreas of Wistar rats) H & E Stain. x 100
Note: D-Duct, BV-Blood vessel

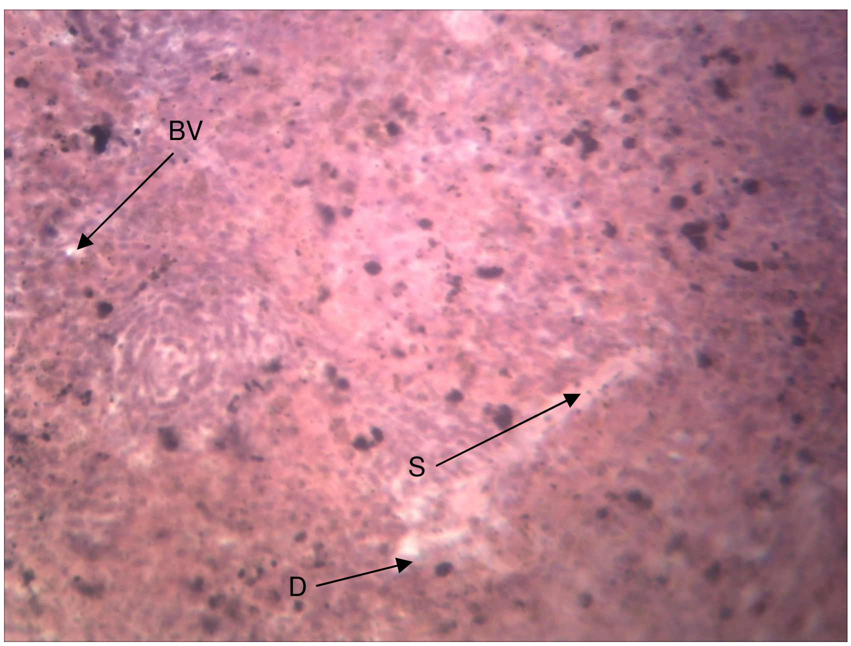

Plate 3: Coronal section of pancreas.
Group 3 (coconut water-treated Diabetic pancreas of Wistar rats for 14 days)
H & E Stain. x 100; NOTE: D – Duct, S – Septum, BV – Blood Vessel

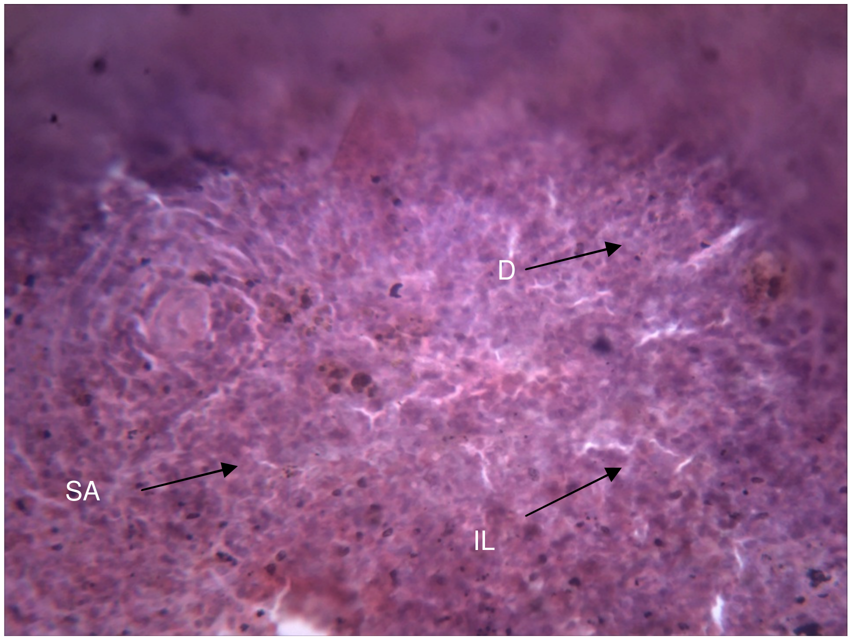

Plate 4 (H & E stain, x100): Coronal section of pancreas.
Group 4 (coconut water-treated Diabetic pancreas of wistar rats for 28 days)
NOTE: SA – Serous Acini, IL – Islets of Langerhans, D – Duct

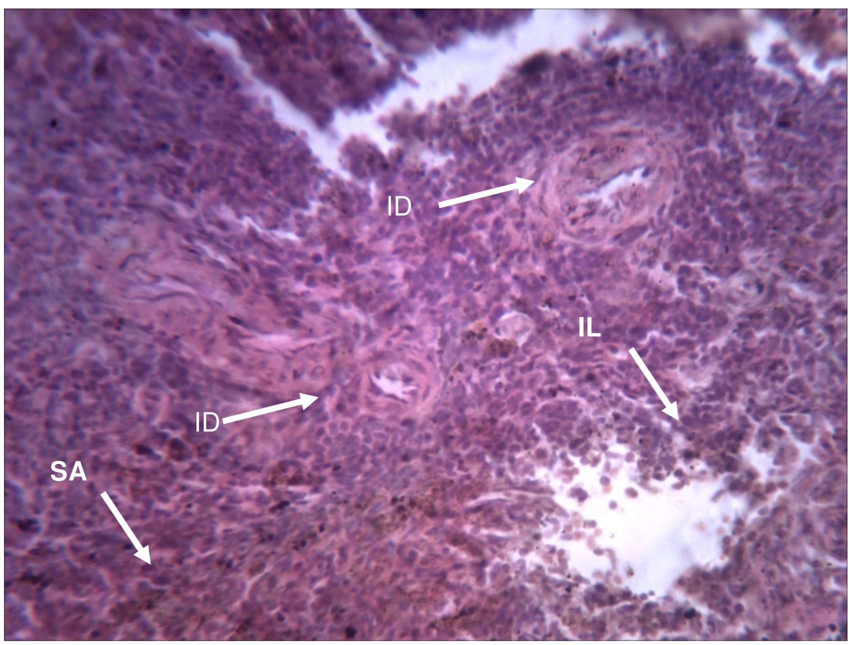

Plate 5 (H & E stain. X100): Coronal section of pancreas:
Group 5 (coconut milk-treated Diabetic pancreas of wistar rats for 28 days
NOTE: IL – Islet of Langerhans, ID – Intralobular Ducts, SA – Serous Acinus,

4. CONCLUSION

This study shows that Coconut water has a blood glucose lowering effect and Coconut milk has a regenerative effect on the pancreatic cells damaged by diabetes. This could be complimentary in the management of our patients especially diabetics.

ACKNOWLEDGEMENTS

The researchers wish to thank Miss Anuli Nwankwo for the preliminary work done in this subject area and to Mrs Julie Nwangwa for proof reading the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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