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Virgin coconut oil dietary consumption ameliorates abnormal haemostatic parameters in diabetic rats

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ABSTRACT

Introduction: Virgin coconut oil (VCO), a saturated fat, has been reported to have anti-diabetic properties and there is dearth of information on its potency to correct abnormal haemostatic parameters associated with diabetic patients.

Objectives: Therefore, this study was carried out to investigate effect of VCO consumption on abnormal haemostatic parameters in diabetic rats.

Materials & Methods: A total of fifteen (15) male rats weighing 200–250g were divided into three (3) experimental groups: Normal control group (Group I) and Diabetic control group (Group II), both fed on normal rat chow for 4 weeks, and the Diabetic test group (Group III) , fed on 10% VCO diet for 4 weeks. The Group II and Group III rats were injected alloxan (150mg/kg) intraperitoneally to induce diabetes. After 72 hours of injection, the blood glucose was determined to confirm hyperglycemia. At the end of the experiment, fibrinogen concentration, prothrombin, bleeding and clotting times were determined in the animals.

Results: The results showed a significant increase in the fibrinogen concentration and prothrombin time of Group II compared to Group I and Group III. However, there was significant decrease in bleeding time of Group II when compared to that of Group I and Group III while the clotting time of Group II was significantly decrease compared to Group I.

Conclusion: Thus, VCO normalizes abnormal haemostatic conditions in diabetics.

Keywords: Virgin coconut oil (VCO), fibrinogen concentration, bleeding time, clotting time, haemostatic parameters, prothrombin time.

INTRODUCTION

Diabetes mellitus is a heterogeneous disease affecting metabolism of various compounds including carbohydrates, lipids and proteins which also impairs biological processes such as coagulation homeostasis that causes vascular thrombotic problems[1,2]. Hyperglycemia, a major feature of diabetes mellitus, has been considered to be the causative factor for abnormalities in coagulation pathways, and exposes patient with diabetes to macrovascular mortality[3]. However, some patients with diabetes mellitus die a thrombotic death. In untreated diabetes mellitus, there is increased thrombotic tendency due to platelet hyper-reactivity and increased activation of prothrombotic coagulation factors coupled with decreased fibrinolysis[4]. In addition, the plasma levels of many clotting factors including fibrinogen[5], factor VII, factor VIII[6], factor XI, factor XII, kallikrein, and von Willebrand Factor are elevated in diabetes[7]. Therefore, the coagulation mechanism of diabetes patients has been a major concern in which even taking of drugs did not have any ameliorating effect except probably dietary consumption of natural products or herbs.

Virgin coconut oil (VCO), a saturated fat, has been reported with favorable anti-diabetic properties. It has been renowned for its nutritional and medicinal value. Studies on the biological effects of coconut oil have shown that it reduces oxidative stress by enhancing the antioxidant defense system, scavenging free radicals and reducing lipid peroxidation[8, 9]. It has also been reported that VCO suppresses microbial and viral activities[10], enhance weight loss and improves thyroid function[11]. In addition, coconut oil possesses anti-inflammatory and anti-ulcerogenic effects[12], and that it has a favorable effect on the lipid profile[8,13]. However, information regarding the effect of virgin coconut oil on altered haemostatic parameters as a result of diabetic condition has not been reported in animal studies. Therefore, in an effort to examine how haemostatic parameters can be controlled in diabetic patients, we examine in this research work, the possible effect of VCO on the fibrinogen concentration, prothrombin time, bleeding time and clotting time in alloxan-induced diabetic male wistar rats.

MATERIALS AND METHODS

2.1. Preparation of VCO

Dry coconuts were bought from Okuku and Bekwarra markets in the northern part of Cross River State, Nigeria. The nuts were broken, and its meat was scrapped from the shell, and cut into a small piece before it was grinded in a grinding machine into viscous substance. Thereafter, the viscous composition was squeezed through a sterile cheese cloth to obtain coconut milk which was kept into glass jars. The glass jars containing the coconut milk were left for not less than 24 hours to allow the coconut milk and the oil to separate into layers. The jars were kept in the refrigerator for 48 hours so that the jar content could harden. Thereafter, the pure virgin coconut oil was separated from the curd which was scooped out from the jar and then discarded. The obtained VCO was in a sterile bottle and stored at room temperature for use in the present study.

2.2. Experimental Animals

Fifteen (15) adult male Wistar rats with a weight range of 200–250g were used in this study. The rats were purchased from the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, Cross River University of Technology (CRUTECH), Okuku Campus, Nigeria. They were kept in wooden cages in the animal house at a suitable temperature and humidity. The rats were given normal rat chow, 10% virgin coconut oil diet (100g of virgin coconut oil mixed with 1000g of normal rat chow) and water ad *libitum* daily. The animals were acclimatized for 2 weeks prior to the start of the experiment.

2.3. Experimental Procedure

Three (3) experimental groups of five rats each were considered in the present study. These include: Group I - normal control group; Group II - diabetic control group (both groups were fed on normal rat chow diet); Group III - diabetic test group (fed on 10% virgin coconut oil diet). The animals in Group II and Group III were induced with diabetes mellitus by single intraperitoneal injection of 150mg/kg of alloxan (Sigma Chemical Company, USA) which was dissolved in normal saline[14]. After 72 hours of alloxan injection, blood samples were obtained from the tail vein of the fasted animals and the animal with a blood glucose level of 250mg/dl (determined by Accu-CHEK Active glucometer) were selected for the study. After the induction of diabetes, the animals in Group I and Group II were given normal rat chow and water ad *libitum* while the animals in the Group III were given 10% VCO diet for a period of 4 weeks. All procedures in the study conformed to the guiding principle for experimental animals as recommended by American Physiological Society guiding principle in the care and use of animals[15].

2.4. Blood Sample Collection

At the end of the experiment, the animals were anesthetized with chloroform and their blood samples were collected through cardiac puncture into EDTA bottles which were centrifuged at 3000g for 10 minutes to obtain sera.

2.5. Determination of Haemostatic Parameters

2.5.1. Determination of Fibrinogen Concentration

Fibrinogen level in the experimental animals was determined by Clauss method[16].

2.5.2. Determination of Prothrombin Time

The prothrombin time was determined as described by Bamidele[17].

2.5.3. Determination of Bleeding Time

This was determined using a modified Dukes method[18]. A disposable lancet blade was used to make a skin puncture in the tail region of the animals. A stopwatch was started as soon as blood appears from the punctured site. The wounded portion was dabbed with a clean filter paper every 15 seconds until the paper is no longer stained with blood. The bleeding time was then taken as the time blood stopped flowing at the punctured site.

2.5.4. Determination of Clotting Time

0.2 ml of blood which was directly taken from the heart was delivered into four glass test tubes that has previously warmed and maintained at 37^0C using a water bath. A stop watch was started immediately the blood was delivered into the glass test tubes. The tubes were continuously tilted at an angle of 90^0 at 40 seconds intervals until the blood in them stop flowing when tilted. The clotting time was taken as the average of the time blood clotted in each of the four test tubes.

2.6. Statistical Analysis

Data were presented as mean ± standard error of mean (SEM). One-way ANOVA and LSD post hoc test were used to determine the specific pairs of groups that were statistically different at $p < 0.05$. Analysis was performed with Statistics Package for the Social Sciences (SPSS) version 16.

RESULTS

3.1. Effect of VCO on fibrinogen concentration

As shown in Figure 1, there was significant difference in the mean value of fibrinogen concentration between the control group (366.50 ± 18.37mg/dl) and the diabetic control group (433.20 ± 17.17mg/dl). Also, the p value was found to be less than 0.05 between the mean value of the diabetic control group and the diabetic test group (372.36 ± 15.82mg/dl). However, there exists no significant difference between the mean value of the fibrinogen concentration of the control group and the diabetic test group.

3.2. Effect of VCO on prothrombin time

The mean prothrombin time of control group was 13.60 ± 1.73sec, while that of the diabetic control and diabetic test groups were 24.40 ± 0.73sec and 15.40 ± 1.31sec respectively as represented in Figure 2. There was significant increase in prothrombin time of diabetic control group compared to that of diabetic test group and control group. On the other hand, there was no significant difference between the mean value of the prothrombin time of the control group and diabetic test group.

3.3. Effect of VCO on bleeding time

Figure 3 showed that there was a significant decrease ($p<0.001$) in the bleeding time of the diabetic control group (204.08 ± 3.89sec) compared to the control group (260.73 ± 5.38sec). This was also the same case in the mean value of the diabetic control group and the diabetic test group (240.36 ± 3.39sec). More so, a statistical significance difference ($p<0.001$) was also established between the bleeding time of the control group and the diabetic test group.

3.4. Effect of VCO on clotting time

The clotting time value expressed as mean ± SEM is depicted in the Figure 4. The figure showed that there was significant decrease ($p<0.05$) in the mean value of the clotting time of diabetic control group (64.80 ± 1.96sec) when compared to control group (78.80 ± 4.20sec). On the other hand, there was no significant difference in the clotting time of diabetic test group (74.00 ± 1.05sec) compared to control and diabetic control groups.

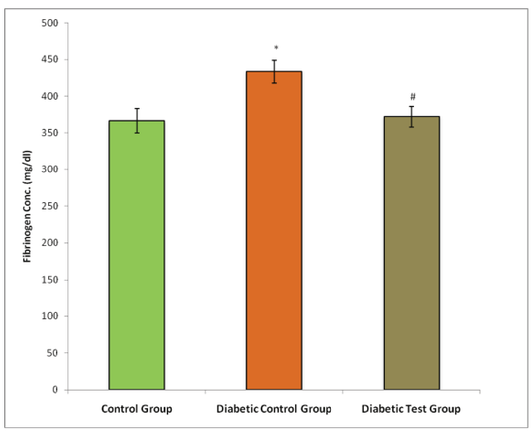

Figure 1: Effect of virgin coconut oil on fibrinogen concentration (mg/dl) in alloxan-induced diabetic male wistar rats. Values are expressed as mean ± S.E.M. *p < 0.05 = Significant compared to control group. #p < 0.05 = Significant compared to diabetic control group.

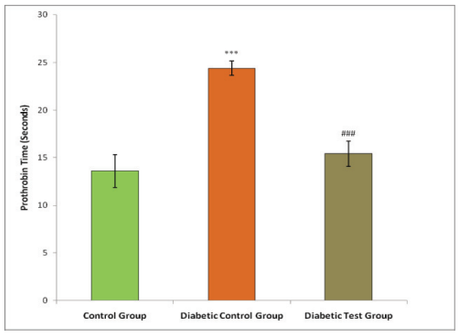

Figure 2: Effect of virgin coconut oil on prothrombin time in alloxan-induced diabetic male wistar rats. Values are expressed as mean ± S.E.M. ***p < 0.001 = Significant compared to control group. ### p < 0.001 = Significant compared to diabetic control group.

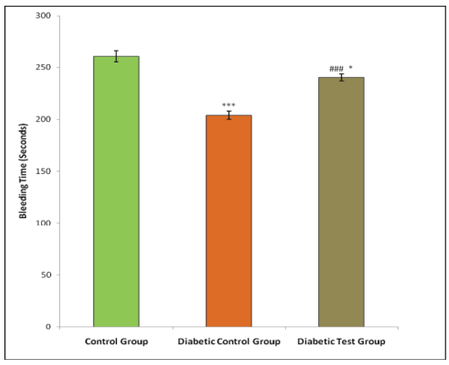

Figure 3: Effects of virgin coconut oil on bleeding time (sec) in alloxan-induced diabetic male wistar rats. Values are expressed as mean ± S.E.M. ***p < 0.001 = Significant compared to control group.. ###p < 0.001 = Significant compared to diabetic control group. *p < 0.05 = Significant compared to the control group

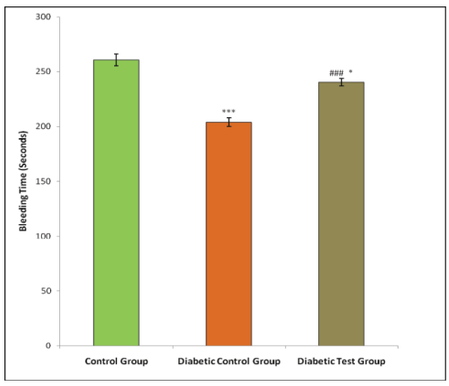

Figure 4: Effects of virgin coconut oil on clotting time (sec) in alloxan-induced diabetic male wistar rats. Values are expressed as mean ± S.E.M. *p < 0.05 = Significant compared to the control group

DISCUSSION

Diabetes mellitus, a chronic disease state, has been reported to feature haemorheological disorders and haemostatic abnormalities[19]. These include; imbalance in fibrinogen concentration, altered prothrombin, bleeding and clotting times, among other important haemostatic parameters. The clotting time measures the intrinsic pathway, the prothrombin time measures the extrinsic pathway of blood coagulation while the fibrinogen concentration is critical to the formation of stable fibrin clot[18].

Plasma fibrinogen levels have been noted to be higher in diabetic patients than in control individual[20]. This has been noted to result to fibrin clot formation, and platelet aggregation[21]. In accordance with these established facts from these previous researchers, we found that the fibrinogen concentration in the diabetic control group was significantly higher compared to that of the control group. It was however noted that VCO brought about a significant decrease in the fibrinogen concentration in the diabetic test group when compared to that of the diabetic control group. There is no doubt that plasma fibrinogen concentration has relationship with the bleeding time and clotting time.

Fibrinolytic activity has been stated to be low in type 2 diabetic subjects. This is thought to be due to high levels of plasminogen activator inhibitor-1, which inhibit the formation of fibrinolytic plasmin from plasminogen[22]. This can be safely attributed to cause the significant decrease in the bleeding time in the diabetic control group. Platelet hyper-reactivity which has been well documented to occur in diabetic subjects can be said to be another contributing factor to the significant decrease in the bleeding time [23].

The activation of extrinsic pathway along side with a decrement in intrinsic pathway of the coagulation cascade in type 2 diabetics has been noted to bring about a reduction in the blood clotting time in diabetic patients[24]. Sauls[21] reported that elevated prothrombin level in diabetics is a potent factor that brings about shortened clotting times. Also, it has been reported that there is an increased markers of coagulation in diabetic subjects[25]. In the present study, it was also observed that the average clotting time in the diabetic control rats was found to be reduced compared to that of the control group and the diabetic test group as expected. In addition, several studies have shown that levels of prothrombin time are increased in both type 1 and type 2 diabetic patients and that glycaemic control normalizes prothrombin time levels in diabetic patients[3]. Therefore, because of the anti-diabetic properties of VCO, it is reasonable to suggest that abnormal haemostatic parameters in diabetic patients can be ameliorated with the control of blood glucose since VCO is anti-diabetic. However, further studies should be done to examine the effect of VCO on other haemostatic parameters.

CONCLUSION

Thus, VCO dietary consumption may therefore be useful for controlling altered haemostatic parameters in diabetes and thrombotic problems.

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