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Prolonged Ingestion of Dietary Cocoa Attenuates Hemoglobin Glycation Associated with Diabetes Mellitus in Rats

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Abstract: Cocoa contains numerous polyphenols with laudable antioxidant activity. Glycation has been implicated in the pathogenesis of complications of diabetes mellitus. We investigate the effect of coca on hemoglobin glycation in rats experimentally induced with diabetes. 21 female Sprague-Dawley rats aged 21 weeks were randomly divided into control "C" and diabetic - diabetics fed cocoa "DC" and diabetics not fed cocoa "D" - groups of 6, 8 and 7 rats respectively. All rats were fed rat chow; C and D groups were fed tap water while the DC group were fed 2% w/v natural cocoa powder in tap water *ad libitum*. Diabetes was induced by a single intravenous shot of streptozotocin (53 mg/kg body weight). Ten weeks post-diabetes, 1ml of blood aspirated from the left ventricle and stored in a plastic tube containing ethylene diamine tetraacetic acid and used to determine the glycated haemoglobin (HbA_{1c}) concentration of each rat using a DCA 2000 plasma analyzer. Data was analyzed using ANOVA and posthoc Bonferroni's Multiple Comparison Test of GraphPad Prism (3.0). Percentage hemoglobin glycation significantly reduced ($p < 0.0001$) reduced from 12.8±0.50 to 7.8±0.27 in D and DC groups respectively but did not attain levels observed in the controls (3.90±0.10). Cocoa powder and other cocoa products can be administered alongside current diabetes management protocols to improve patients' outcome.

Key words: Diabetes mellitus · Cocoa · Hemoglobin glycation · Attenutation

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder with a high prevalence and an increasing global incidence. It is characterized hyperglycemia (plasma glucose ≥ 11 mmol/L), hyperlipidemia, polydipsea, polyurea amongst others [1]. Hyperglycemia is still considered the principal cause of complications in DM [2]. These complications have been attributed to the markedly accelerated formation of sugar-derived substances known as advanced glycation end products (AGEs) [3].

The initial nonenzymatic reaction of glucose with free amino groups of proteins, nucleic acids and lipid of tissues (glycation) cause reversible Schiff base formation which are spontaneously rearranged into Amadori products including Hemohlobin A1c (HbA1c). Persistently high glucose levels (as seen in DM) lead to irreversible glycation and AGE formation. The extent of glycation is proportional to glucose concentration and it is associated with oxidative stress induced by AGE interaction with receptors on tissues [2].

DM-related glycation has been implicated in cataract and retinopathy, nephropathy, neuropathy and hepatitis [2, 4]. Previous studies have noted that reducing glycation (and associated oxidative stress) in the long term management of DM can be achieved by administering antioxidants [5].

Cocoa is a polyphenol whose antioxidant action has been intensely investigated [6, 7, 8]. It has been found to have nearly twice the antioxidant content of red wine and up to three times that of green tea [9]. Flavonoids from cocoa are most beneficial in reducing cardiovascular risk (including DM) compared to those from other polyphenols [10]. Other studies have confirmed that Cocoa has anti-inflamatory, anti-hypertensive, anticarcinogenic effects [10, 11, 12] in addition to its

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ability to boost insulin production and sensitivity, [13] and reversing vascular dysfunction in diabetes by improving vasodilation of the brachial artery [14] amongst others. Since AGE has been implicated as being pro-inflammatory and highly oxidative, we seek to investigate the effect of antioxidant-rich cocoa on hemoglobin glycation of experimentally-induced diabetes in rats.

MATERIALS AND METHODS

The University of Ghana Medical School (UGMS) ethical committee approved this work. Twenty-one female rats aged 21 weeks with average weight of 200g, were kept under 12 hour alternating periods of light and darkness at 25°C in the UGMS animal house. The rats were randomly divided into 3 groups: control (C), diabetic rats fed with cocoa (DC) and diabetic rats not fed cocoa (D) containing 6, 8 and 7 rats respectively. All rats were fed with rat chow. The C and D groups were given tap water whilst the DC group had 2% w/v natural cocoa powder (Goodfood brand) in tap water (prepared according to manufacturer's instruction). Weights of the rats and fluid consumed were measured over the experimental period.

Experimental Procedure: Diabetes was induced by giving the rats a single tail vein injection of streptozotocin (STZ; 53 mg/kg, I.V., n=15) in 0.1 M citrate buffer. Rats serving as controls were given an equivalent volume of a single injection of 0.1 M citrate buffer. Diabetes was confirmed by blood glucose estimation (Glucometer Ascencia Contour, Biomol Corporation ®, USA) 2 days post-STZ injection. Plasma glucose level ≥ 11 mmol/l (200 mg/dl) [15] confirmed diabetes mellitus. Insulin supplementation was not given and a seven-day period was allowed for the rats to adjust to the diabetic condition. The duration of the experiment after the induction of diabetes was ten weeks.

1ml of blood aspirated from the left ventricle was stored in a plastic tube containing ethylene diamine tetraacetic acid (EDTA) and used to determine the glycated haemoglobin (HbA_{1c}) concentration of each rat using a DCA 2000 plasma analyzer (Bayer diagnostics, Germany).

Statistical Analysis: The results were analyzed using analysis of variance (ANOVA) and followed with posthoc Bonferroni's Multiple Comparison Test of GraphPad Prism 3.0) to reveal actual differences. The results are presented as tables and charts.

RESULTS

Significant differences existed in the percentage hemoglobin glycation within the three groups of rats ($p < 0.0001$). Bonferroni Multiple Comparison Test for percentage glycation showed differences in the C vs DC ($p < 0.0001$), C vs D ($p < 0.001$) and DC vs D ($p < 0.001$) groups of rats (Fig. 1 and 2 below). There were 2 - fold, 3.3 - fold and 1.67 - fold increase in the C to DC, C to D and DC to D groups of rats respectively. The glycation in DC vs D group showed a reduction in the former by 0.39%.

DISCUSSION

The purpose of this study was to investigate the effect of prolonged cocoa powder ingestion on the percentage hemoglobin glycation in diabetic rats.

The percentage hemoglobin glycation indicates the extent of tissue and cellular oxidation by the reactive oxygen species (ROS). It increases in DM [16]. The interpretation of HbA1c values in the management of diabetes are: values below 7% is good, 7%-9% is average and above 9% poor control [17]. It represents average glycemia control over a period of six - eight weeks [18].

Reduction in HbA1c in experimentally-induced DM was observed in this work. We attribute this to regular ingestion of dietary cocoa. Cocoa can attenuate the devastation of oxidative stress by its antioxidant action [3].

Ingestion of cocoa powder improved the glycation in diabetic rats from and extremely poor value (12.8%) to a promising average value (7.8%). It is also noteworthy that diabetic rats regularly fed cocoa had the extent of their glycation lowered by as much as 0.39% relative to their counterparts not fed cocoa. Other studies have also observed a reduction in HbA1c with cocoa [19], green tea [20], vitamin C and E [21], miglitol [22] and cinnamon [23]. Our observation was similar to HbA1c among diabetic patients who serially monitored their blood glucose [21]. Hence both can be harnessed to improve glycation control.

Since glycated hemoglobin is a predictor of diabetes complications, a reduction in HbA1c - like we observed in this study - can reduce the risk of micro and macrovascular diseases whose impact are on the increase in DM. Advocating cocoa supplementation alongside present management protocols for DM may help improve patient outcomes.

Fig. 1: Percentage glycated hemoglobin 10 weeks post-diabetes

	C	DC	D
HbA_{1C} (%)	3.90±0.10	7.8±0.27*	12.8±0.50*

* P value < 0.05 was considered to be statistically significant

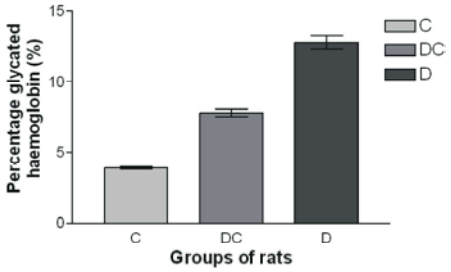

Fig. 2: Plasma HbA1c within the 3 rat groups HbA1c levels in the 3 groups of rats differ significantly from one other

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