Original Article

Improvement in nutrient handling in STZ induced diabetic rats treated with *Ocimum gratissimum*

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Abstract

Objective: Alteration in digestive and absorptive enzymatic activities has been reported in diabetes mellitus (DM), but not with *Ocimum gratissimum* (OG) treatment. This study was, therefore, designed to indirectly assess the effect of DM and treatment with OG on nutrient digestion and absorption, through estimation of their fecal excretion. **Materials and Methods:** Animals were randomly assigned into three groups of six per group for control, DM and diabetic mellitus treated (DMT). Diabetes was induced by single intraperitoneal injection of 65 mg/kg streptozotocin in the test groups. OG was administered to the DMT group at dose of 1500 mg/kg once daily for 28 days. Fecal glucose, protein and cholesterol were determined. **Results:** Fecal glucose was significantly ($P < 0.001$) lower in the DM group compared to the control and DMT groups, with the DMT groups significantly ($P < 0.001$) lower than the control. Fecal protein was significantly ($P < 0.001$) lower in the DM group than the control whereas it was significantly lower in the DMT groups than the DM. Fecal cholesterol was significantly ($P < 0.001$) higher in the DM than the DMT and control groups with DMT significantly ($P < 0.01$) higher than the control. **Conclusion:** This result indicates the propensity of OG to reverse impairment of nutrient digestion and absorption in DM.

Key words: Absorptive enzymes, digestive enzymes, fecal cholesterol, fecal glucose, fecal protein, *Ocimum gratissimum*

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Introduction

Transport of chyme through the intestine is closely linked to intraluminal digestion and absorption of nutrients. The efficacy of absorption of nutrients is, therefore potentially affected by dysmotility of the small intestine observed in DM,[1] and by alterations in the transport mechanisms facilitating nutrient uptake across the intestinal membrane.[2]

Furthermore, changes in intestinal mucosal function were observed in diabetic rodents but are unclear whether these are intrinsic and contributory to the disease process or secondary to the disease. For example, the bio-breeding (BB) rat exhibits enzymatic glycosylation of the intestinal brush border enzyme, amino-oligopeptidase, soon after the onset of DM, with a reversal of this pattern with vigorous insulin treatment. In contrast, this is not evident in the STZ rat,[3] which typically has less severe DM. A similar change and reversal in the BB rat has also been observed with intestinal sucrose-x-dextrinase.[4] Large increases in intestinal acyl-coenzyme A (CoA): Cholesterol acyltransferase and cholesterol esterase, implicated in cholesterol absorption from the gut, were also observed in the STZ diabetic rat, and these elevations were reversed with insulin-mediated improvement of glycemic control.[5] It thus appears likely, from these studies that diabetes-associated changes in gut enzyme expression represent a response to some aspect of the diabetic state, since they occur in both chemically-induced and genetic models, and are reversible with rigorous treatment of the DM.

Experimental diabetes in animals has been reported to enhance glucose absorption and increase glucose metabolism.[6] The increase in glucose absorption in the enterocytes[7,8] is accompanied by an increase in the

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expression of glucose transporters SGLTI and GLUT2 and their mRNAs in diabetic rats and humans.[8,9]

There is little evidence that DM *per se* affects protein absorption to a clinically relevant extent.[10] However, when DM is associated with pancreatic insufficiency, coeliac disease or bacterial over-growth, mal-absorption of protein may occur.[11] In diabetic patients with bacterial over-growth, protein mal-absorption is the result of several factors: The abundantly present bacteria compete with the host for protein; amino acid absorption may be impaired as a result of mucosal damage, and the levels of proteolytic enzymes may be decreased.[12,13]

Since lipid absorption is dependent on the interplay of several organs (small intestine, liver, gallbladder, pancreas), DM has the potential to be associated with fat mal-absorption, although there is a substantial functional reserve that compensates for minor changes in some of the critical phases of this process.

Fat mal-absorption may also result from loss of effective absorption surface, as in diabetic patients with co-existent celiac disease. Dysmotility associated with bacterial over-growth can also lead to mal-absorption.[14,15] The bacterial deconjugation of bile acids is the primary mechanism for mal-absorption of fats and fat–soluble vitamins; anaerobic organisms in particular reduce the level of conjugated bile acids below the critical micelle concentration leading to steotorrhae.[16]

Phytochemical analysis of *Ocimum gratissimum* (OG) revealed important constituents as tannins, alkaloids, saponins, flavonoids and phenolic compounds.[17] OG has been reported to alleviate derangements in serum and biliary bilirubin, cholesterol, and electrolytes in streptozotocin-induced diabetic rats.[18] The hypoglycemic properties of OG claimed by traditional herbal medicine practitioners had been investigated and confirmed to be true.[19] Therefore, the need to assess the effect of OG on other gastrointestinal indices related to the pathogenesis of DM becomes cogent.

The quantity of fecal glucose, protein and cholesterol could be an indirect indicator of the level of activity of their respective enzymes. Alteration in these absorptive enzyme activities has been reported in DM. This study, therefore, aims to provide useful information on the effect of DM on these nutrients absorption with a chance of fresh findings on how OG may alter the status quo, at least by indirect deduction.

Materials and Methods

Plant materials and preparation of aqueous extract

The leaves of OG were obtained from the University of Calabar Botanical Garden and identified by the Chief Herbarium Officer of Botany Department of the University of Calabar. The fresh leaves were rinsed with water to remove sand and debris and then allowed to air dry. The leaves were then dried under shade for 2 days and then transferred into AstellHearson Oven and dried at a temperature range of 40°C–45°C.

The dried leaves were then ground in an electric blender into a fine powder to give a gram weight of 527 g. This 527 g weight was soaked in 2.65 L of water (distilled water) and was soaked overnight for about 15 h and stirred at regular intervals. The mixture was filtered using a satin mesh material, and the final filtrate was obtained using Whatman's filter paper size 1. The final filtrate was dried in the AstellHearson Oven at 45°C to obtain a brown gummy paste. A mettler P163 electronic weighing balance was used to weigh the gummy paste before stock solution was prepared. The stock solution of the extract was prepared by dissolving 15 gm of extract in 10 ml of water to give a concentration of 1500 mg/ml. The stock solution was labeled appropriately and refrigerated at 4°C until required for use. The median lethal dose (LD_{50}) of the plant extract was determined by method of Lorke.[20]

Determination of phytoconstituents

The phytoconstituents of the extracts was determined and were screened for the presence of carbohydrates, tannins, alkaloids, saponins, phenolics, anthraquinones and cardiac glycosides as described by Trease and Evans[21] and Sofowora.[22]

Animals preparation, experimental groupings and treatment

Eighteen male albino Wistar rats were used for the study, the animals were divided into three groups and were assigned randomly into each group that was made up of six (6) rats each and housed in cages assigned to them.

The first group was made up of the control animals, which were fed with normal rat chow (feed). The second group contained streptozotocin induced diabetic rat, which were left untreated. A third group of animals contained the test group, which was streptozotocin induced diabetic rats treated with aqueous leaf extract of OG. The experimental procedures involving the animals and their care were in line with the approved guidelines by the local (University of Uyo, Akwa Ibom State) research and ethical committee established and guided by the Helsinki Declaration on Animal research.

Induction of diabetes mellitus

Type I diabetes mellitus (DM) was induced in 12 male albino Wistar rats by a single injection of 65 mg/kg streptozotocin. The injection was given intraperitoneally. The state of diabetes was observed after 48 h by the symptoms of polyuria and glucosuria, and this state was confirmed using uristic test strip (Bayer

Health Care, LLC, USA). Also, the blood glucose level was tested 1-week after induction using a Glucometer (ACCU-CHECK Advantage II, Roche Diagnostics (GmbH, Germany) and ACCU-CHECK Advantage II test strips.

Extract administration and observation

One week after induction of diabetes in the 12 male albino Wistar rats, the extract was administered per oral to the diabetic mellitus treated (DMT) group at a dose of 1500 mg/kg body weight daily for 28 days. Administration was facilitated by the use of a syringe and Orogastic tube.

Determination of total fecal lipids

Total frozen dry fecal matter from each rat was ground with a mortar and pestle into a homogenous mixture. Fecal lipid was extracted using a modification of the method of Folch *et al*.[23]

Determination of fecal protein and glucose

A total of 1 g of fecal material was collected from all the groups. The fecal matter from each group was weighed and put into a small bottle. Fifteen ml of distilled water was then added and left for 30 min. The homogenate was filtered using Whatman number 1 filter paper. The supernatant was then transferred into plastic containers and refrigerated and used for analysis of fecal protein and glucose.

Fecal protein estimation

Estimation of fecal protein was conducted using Biuret method.[24]

Fecal glucose estimation

The Dialab method of glucose estimation was employed.[25] In the presence of glucose oxidase, glucose is oxidized to glucoronic acid and hydrogen peroxide. The hydrogen peroxide reacts in the presence of peroxidase with phenol and 4-amino phenozone to form a quinone dye (pink) whose color intensity is in proportion to the glucose concentration in the sample.

Statistical analysis

All results were presented as mean + standard error of the mean. Three sets of data were analyzed using one-way ANOVA, followed by the least significant difference procedure for significant $P < 0.05$ was considered as significant. Computer software SPSS version 18.0 by SPSS Incorporated, Chicago was used for the analysis.

Results

Fecal glucose estimation in the control, diabetic mellitus and diabetic mellitus treated groups of rats

The mean values were: 0.68 ± 0.018, 0.356 ± 0.021 and 0.505 ± 0.013 mmol/L in control, DM and DMT groups, respectively. The DM values was significantly lower ($P < 0.001$) than the control and the DMT groups, with the DMT significantly ($P < 0.001$) lower than the control as shown in Figure 1.

Fecal protein estimation in the control, diabetic mellitus and diabetic mellitus treated experimental groups of rat

The average values were: 9.783 ± 0.047, 8.966 ± 0.12 and 7.166 ± 0.066 mmol/L for control, DM and DMT groups respectively. The DM group was significantly lower ($P < 0.001$) than the control and the DMT was significantly lower ($P < 0.001$) than the DM group [Figure 2].

Fecal cholesterol estimation

The mean values were: 0.313 ± 0.004, 0.491 ± 0.015 and 0.383 ± 0.016 mmol/L for control, DM and DMT groups respectively. There were significant differences between the test groups and the control. The DM group was significantly higher ($P < 0.001$) than the DMT and control groups. The DMT was significantly higher ($P < 0.01$) than the control [Figure 3].

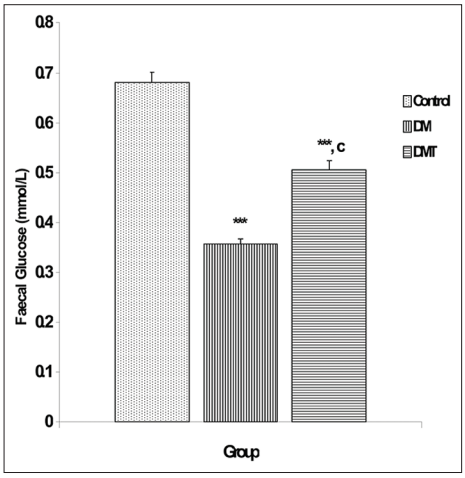

Figure 1: Comparison of fecal glucose level in the different experimental groups $n = 6$. ***$P < 0.001$ versus control, c = $P < 0.001$ versus diabetes mellitus

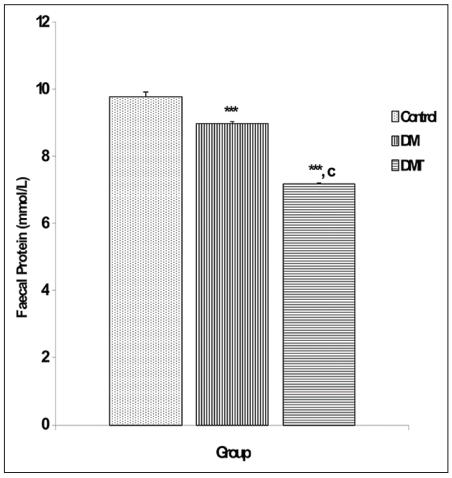

Figure 2: Comparison of fecal protein concentration in the different experimental groups $n = 6$. ***$P < 0.001$ versus control, c = $P < 0.001$ versus diabetes mellitus

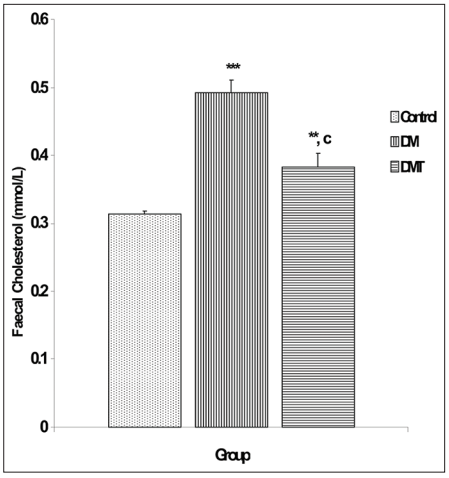

Figure 3: Comparison of fecal cholesterol concentration in the different experimental groups $n = 6$. ***$P < 0.001$ versus control, c = $P < 0.001$ versus diabetes mellitus

Discussion

Fecal glucose was reduced in the DMT and DM groups compared to the control, with the DMT significantly higher than the DM group. Previously,[26] it has been commented that it is possible that bacteria in the colon may ferment the largely undigested and unabsorbed carbohydrate to other products other than glucose. In support of this proposition, a study[27] reported bacterial fermentation of carbohydrates. The markedly increased fecal glucose in the DMT group compared to the DM group could likely be attributed to the possible antibacterial action of OG. Several studies had previously reported the efficiency of OG extract and its essential oil (EO) as an antibacterial agent. For example, Celso *et al*.[28] had showed that Gram-negative bacteria belonging to the genera *Proteus*, *Klebsiella*, *Salmonella*, *Escherichia* and *Shigella* were inhibited by EO of OG with minimal inhibitory concentrations (MICs) ranging from 3 to 12 μg/ml. Other reports have shown MIC results similar to or higher than this.[29] Reduced bacterial load in the gut limits the extent of carbohydrates fermentation into other products in the DMT group. The differences in the MIC in the various reports were explained on the basis of susceptibility testing conditions, physicochemical characteristics of the oil and even strain to strain differences.

The fecal protein in the DM and DMT groups were significantly less than the control group, with the DMT group lesser compared to the DM group. Empirically, this shows that DM and treatment with OG could enhance protein digestion and absorption. There is little evidence that DM *per se* affects protein absorption to a clinically relevant extent.[10] It has also been reported that when DM is associated with severe pancreatic insufficiency, coeliac disease or bacterial over-growth, mal-absorption may occur.[10] In diabetic patients with bacterial over-growth, protein mal-absorption is the result of several factors: The abundantly present bacteria compete with the host for proteins, amino acid absorption may be impaired as a result of mucosal damage, and the levels of proteolytic enzymes may be decreased.[12,13,30] The above pathologic processes would result in increased fecal proteins, but this was not the case in this study. It is, therefore, likely that other pathophysiological events could have contributed to this deviant occurrence. Thus, it is inferred that fecal protein content may not be solely dependent on the efficiency or otherwise of protein digestion and absorption process after all.

Fecal cholesterol in the DMT and DM groups were raised compared to the control group. The DMT group was however lower, when compared to the DM group. This finding is in line with reports that DM is associated with increased cholesterol secretion. Since lipid absorption is dependent on the interplay of several organs, (small intestine, pancreas, liver, gall bladder) DM has the potential to be associated with fat mal-absorption. It has been observed that the functional capacities of these organs may have been compromised one-way or the other from our results in this study. Although it is not known whether impaired small intestinal motility *per se* can lead to fat mal-absorption, it certainly can when the impaired motility is associated with bacterial over-growth.[14,15] The above predisposing factors, (dysmotility and bacterial over-growth) may be implicated in the deranged cholesterol levels in the DMT and DM groups. Again, the antibacterial action of OG tends to ameliorate this derangement as seen in the significant reduction in the fecal cholesterol levels in the DMT group compared with the DM group.

Specifically, it is most likely that OG might have inhibited the activity of intestinal acyl-CoA, cholesterol transferase and cholesterol esterase responsible for intestinal cholesterol absorption, thus the excretion of excess cholesterol in the feces. It has been reported that diabetes associated changes in gut enzyme expression represent a response to some aspect of the diabetic state since they occur in both chemically induced and genetic models, and are reversible with vigorous treatment of DM.[5] There appears to be a multi-factorial mechanism responsible for the alteration in the fecal excretion of glucose, protein and cholesterol in the diabetic state. DM tends to cause a reduction in fecal glucose and protein excretion, while increasing fecal cholesterol excretion.

Conclusion

Altered nutrient absorption in DM was adjusted toward normal with OG treatment except for protein. This is an

indirect expression of the efficacy of OG to reverse some adverse effect of DM on nutrient digestion and absorption.

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