Full Length Research Paper

Modulation of amylase activity, a possible mechanism of the hypoglycemic effect of ginger (*Zingiber officinale*) extracts in rats

*Paulina Oludoyin Adeniyi[1,2] and Rasaki Adegoke Sanusi[1]

[1]University of Ibadan, Ibadan, Nigeria.
[2]Institute of Agricultural Research and Training, Apata, Ibadan, Nigeria.
*Corresponding author email: doyinadeniyi@yahoo.com

ABSTRACT

The attempt to overcome the limitations of the commonly used anti diabetic drugs in order to combat diabetes mellitus effectively has drawn the attention of researchers to ascertaining the possible use of spices in the prevention and management of this metabolic syndrome. Different extracts of ginger have been reported to exert hypoglycemic effect but for proper application of this concept there is need to understand the mechanisms of action of this effect especially in type 2 diabetes. This study hereby determined the effect of raw and cooked ginger juice on pancreatic amylase activity in normal and high fat diet-induced diabetic rats as it affected the acute and chronic postprandial blood glucose. Eleven weeks old male albino rats (63) were divided into seven groups and treated thus: NS- normal (negative) control, NR- normal rats given raw ginger juice, NC- normal rats cooked ginger juice, DS- diabetic control group, DR- diabetic rats given raw ginger juice, DC- diabetic rats given cooked ginger juice and DD- diabetic rats given Metformin (an anti diabetic drug). The normal and diabetic groups were fed normal rat pellets and high fat diet (HFD) for 12 weeks respectively after which the extracts were administered orally for 4 weeks. The acute and chronic effect after starch and extracts load were determined, The pancreatic amylase activity was determined using Abnova Amylase Activity Assay kit. Data were expressed as mean standard deviation while ANOVA and LSD were used in comparison. The HFD(High Fat Diet) protocol rendered the animals diabetic (Fasting Blood Glucose > 170mg/dl) while the extracts increased hepatic blood glucose significantly (p<0.05) after starch load in the chronic effect. I n the normal groups raw extract increased pancreatic amylase activity (PAA) by 1.1% but the cooked extract reduced it by 25%. Raw, cooked extracts and the drug reduced the PAA by 17, 36 and 49% respectively in the diabetic groups though this did not alter glucose output into the blood in the chronic effect. Since glucose output into the blood was not influenced by the change it the PAA(Pancreatic Amylase Activity) , the intermediate absorption process could be a probable interfering determining factor.

Keywords: Raw ginger, cooked ginger, amylase activity.

INTRODUCTION

The exploration of the possible use of ginger in the dietary management of diabetes mellitus is a laudable intervention long overdue. This is because the hypoglycemic and anti diabetic effect of different extracts and forms of the spice have been ascertained by very many studies in both laboratory animals and human subjects in the two types of diabetes (Arablou et al.,2014; Mozaffari-Khosravi et al., 2014; Son et al., 2014; Mahluji et al., 2013; Sukalingam et al., 2013: Abdulrazaq et al., 2012; Jafri et al., 2011; Iranloye, 2011; Morakinyo, 2011; Elshater et al., 2009; Al-Amin et al.,2006; Ojewole 2006; Akhani et al 2004) but there is an abject scarcity of reports on cooked ginger extract. This is a prominent deficit in knowledge since ginger is mostly consumed in cooked form in various cuisines such as stews, soups, sauces, sweet and savoury puddings, grills, roasts etc.

More still, the safety of its use during pregnancy and lactation as well as the little or no adverse effects (commonly associated with most anti diabetic drugs) makes ginger a possible effective and preferred alternative. Metformin has been identified to exert the following side effects: gastrointestinal disorder such as anorexia, diarrhea, dyspepsia, flatulence, nausea and vomiting; metallic taste; anaemia; lactic acidosis; and reduced absorption of vitamin B12 (Diabetamin hovid, Malaysia) while the safety of its use during pregnancy and lactation has not been ascertained. Glibenclamide may also exhibit the following side effects: gastrointestinal disorders (stomach discomfort, nausea, diarrhea), hepatitis, jaundice, skin itching and rashes, porphyria, abnormal biological tests results that have to do with liver, kidney or blood (Sanofi aventus Daonil glibenclamide- Swiss Pharmacy, Nigeria). Hence there is need for the anti diabetic effect of this spice to be harnessed maximally for effective combat against the continual increasing global prevalence of diabetes.

Understanding the mechanisms of action of the hypoglycemic effect of ginger is a necessity for the proper application of its use. The major metabolic pathways contributing to the lowering of blood glucose include: delayed release of glucose from the digestive system into the blood; increased peripheral glucose utilization mediated by increased insulin secretion (in Type 1 diabetes) and improved insulin sensitivity; and inhibition of hepatic production of glucose via gluconeogenesis (or glycogenolysis). Many hypoglycemic agents exert the effect via one or more of these pathways. Pancreatic amylase is a major enzyme involved in carbohydrate digestion, hence, the modulating effect of ginger on its activity needs to be ascertained.

In vitro studies have reported a slight or significant inhibitory effect of ginger extracts on α-amylase (Moss-Pierce et al., 2013; Akinyemi et al., 2010; Ranilla et al.,2010) while in vivo studies observed a significant increase in the activity of the enzyme by ginger in rats Nwachuckwu and Ohiri 2012; Platel and Srinivasan, 2000).

The conflicting nature of these reports coupled with the need to ascertain the effect of cooked ginger extract on α-amylase (since the spice is mostly consumed in cooked form in various cuisines) demands study in this area. Hence, this protocol determined the effect of raw and cooked ginger juice on pancreatic amylase activity in normal and high fat diet-induced diabetic rats.

MATERIALS AND METHOD

Extracts preparation

Ginger rhizhomes were purchased from Bodija market in Ibadan, Nigeria. The raw extract was prepared according using the method of Elshater et al., 2009 with slight modification. Ginger rhizomes was washed, weighed, peeled, weighed and wet-milled using plate attrition mill (Amuda mill, India). The smooth slurry was sieved using cheese cloth the raw extract was stored in plastic jars at $2^{o}C$ until use.

Cooked ginger was prepared by boiling the raw ginger extract for 1 hour on the medium burner of a 3-burner Thermocool gas cooker, India. This was allowed to cool and stored in a plastic jar at $2^{o}C$ until use.

Formulation of high fat diet (HFD)

High fat diet was formulated using the composition as follows: 45% normal rat pellets, 30% beef tallow, 20% full cream milk powder and 5% sugar (Panchal et al., 2011).

Collection of rats

Male Albino rats (63) of weight range 120-160g were purchased from the animal house of the Department of Veterinary Physiology, University of Ibadan. The rats were fed rat pellets and tap water ad libitum for the period of acclimatization. All animals were treated in accordance with the experimental protocol approved by the Ethical Review Committee of the University of Ibadan/ University College Hospital, Ibadan, Nigeria.

Experimentation

The groups of rats as designated were treated thus: NS – rats fed with normal rats pellets for 12 weeks and 2ml/kg body weight distilled water orally for the following 4 weeks, NR - rats fed with normal rats pellets and given oral administration of raw ginger extract (2ml/kg body weight) for 4 weeks, NC- rats fed with normal diet and given cooked ginger extract, DS- rats fed with HFD and given distilled water, DR - rats fed with HFD and given raw ginger extract, DC - rats fed with HFD and given cooked ginger extract and DD - rats fed with HFD and given Metformin, Diabetamin, Malaysia,180mg/kg body weight (Zheng et al, 2012).

Confirmation of diabetes

After the 12 weeks diet regimen blood was taken from overnight fasted rats in all groups via the tail end and the blood glucose was determined using ACCUCHEK Acute glucometer, Roche, Germany to ascertain normoglycemia in the normal groups and stable hyperglycemia for diabetes (Fasting Blood Glucose ≥ 170mg/dl) in the diabetic groups.

Acute and chronic effect

The method of Tomo et al., 2004 was used with slight

Table 1- Acute effect of ginger extracts on FBG after a starch load

Group	BG/SD 0MIN	BG/SD 30MINS	BG/SD 60MINS	BG/SD 90MINS	BG/SD 120MINS	BG/SD 150MINS	BG/SD 180MINS
NS	110,15/2.83	126.73/4.33	141.52/0,71	135.42/2.11	121.04/1.44	117.52/2.12	110.15/6.4
NR	110.53/5.66	119.05/1.53	128.81/4.02	122.05/0.07	117.53/2.12	116.13/2.83	110.32/0.78
NC	112.54/3.54	127.57/9.19	143.06/7.17	126.13/4.22	117.29/3.54	111.08/9.90	112.58//1.71
DS	185.22/4.24	200.56/4.95	235.5/2.12	217.54/5.66	210.05/1.05	197.30/1.77	189.08/1.88
DR	186.08/1.41	197.19/1.62	215.04/0.32	210.18/1.31	201.43/9.90	191.12/5.66	182.45/5.54
DC	183.55/0.71	197.58/7.12	219.90/7.78	221.51/6.33	206.84/4.95	195.50/6.36	188.33/7.12
DD	185.28/4.24	185.15/5.66	218.52/3.54	215.15/8.13	206.04/6.97	192.52/3.44	186.42/4.85

FBG- fasting blood glucose
BG/SD- blood glucose ± standard deviation

Table 2. Chronic effect of ginger extracts on FBG after a starch load

Group	BG/SD 0MIN	BG/SD 30MINS	BG/SD 60MINS	BG/SD 90MINS	BG/SD 120MINS	BG/SD 150MINS	BG/SD 180MINS
NS	111.03/2.83	119.04/2.41	130.61/2.14	121.23/1.71	117.05/1.47	103.53/2.17	107.05/9.88
NR	83.12/1.45	97.60/2.17	105/55/0.72	120.53/3.54	113.51/2.16	104.35/0.75	92.53/7.87
NC	84.40/4.24	100.38/2.44	128.03/1.48	131.53/1.45	125.53/2.22	115.56/2.52	102.53/3.52
DS	215.22/1.32	238.11/1.78	255.14/5.66	242.03/1.44	229.33/2.16	222.01/4.27	214.61/4.93
DR	115.10/1.56	125.36/3.54	138.51/0.79	145.33/2.17	132.55/0.78	123.57/3.55	118.04/1.47
DC	140.52/2.83	152.66/4.85	161.56/4.95	168.02/1.24	155.56/2.11	147.52/7.78	149.03/5.66
DD	115.28/2.12	128.34/1.33	133.43/2.45	142.28/4.34	129.51/2.54	122.08/8.51	114.59/4.97

FBG- fasting blood glucose
BG/SD- blood glucose ± standard deviation

modification. Blood glucose of overnight fasted rats were recorded after which starch and extracts were given at 2g/kg body weight and 2ml/kg body weight respectively with a feeding cannula. Blood glucose was then recorded at 30, 60, 90, 120, 150 and 180 minutes. For the chronic effect this was repeated after 4 weeks' extracts administration as designated after which the animals were sacrificed via cervical dislocation and the pancreas were carefully removed for amylase activity assay.

Pancreatic amylase activity assay

The amylase activity was determined using Abnova Amylase Activity reagent kit, Taiwan. Tissue homogenate was prepared by crushing 1g of pancreas with 5ml of cold PBS in ice cold mortal and pestle. This was then centrifuged at 10,000rpm for15 minutes at 4oC. Into 1.5ml eppendorf tubes 20µl of supernatant was measured after which 380µl of Substrate was added. This was vortexed briefly and incubated for 5 minutes at 37oC after which 40µl of the stop solution was added and mixing was done by vortexing. This was centrifuged at 14,000rpm for 5 minutes after which the optical density was read at 595nm. For blank the sample was replaced with distilled water. The optical density of blank, distilled water and the calibrator were also read at the wavelength. The amylase activity was calculated as follows (OD- Optical density):

$$\text{Amylase activity (U/L)} = \frac{\text{OD sample} - \text{OD blank}}{\text{OD cal} - \text{OD water}} \times 550$$

Statistical analyses

All data are expressed as mean ± standard deviation. Comparison between the groups was done using Analysis of Variance while Least Significant Difference was used to compare each group with others ($p < 0.05$).

RESULT AND DISCUSSION

Fasting blood glucose (FBG)

There was no significant difference in FBG (BG/SD 0MIN) in all the groups before the introduction of the HFD. The consumption of HFD   for 3 months increased the fasting blood glucose (FBG) from 111.07mg/dl in the normal groups to 185.03 in the diabetic groups (Table 1). This corroborates past findings that reported induction of hyperglycemia (fasting blood glucose ≥ 170 mg/dl) with chronic high fat diet consumption (Magao et al., 2012; Zong et al., 2012; Akerfiedt and Laybut, 2011; Panchal et al., 2011).

Acute effect

The postprandial blood glucose reached its peak value at

Table 3. Effect of raw and cooked ginger extracts on pancreatic amylase activity

Groups	Amylase activity (U/L)
NS	833.39 ± 1.47
NR	842.95 ± 1.05
NC	624.55 ± 3.24
DS	813.36 ± 1.13
DR	672.44 ± 1.38
DC	524.44 ± 3.12
DD	412.19 ± 0.86

60 minutes in all groups while there existed significant difference (p< 0.05) in the rate of this increase between all the groups (Table 1). This was in line with the reports of Tomo et al., 2004 who observed a peak value in blood glucose at 60 minutes after oral administration of starch and amylase inhibitor but in the negative control group the peak value, though higher than the experimental group, was observed at 50 minutes. This shows that postprandial blood glucose is highest at 1hour after the diet load and reduces as the body needs requires and this is not altered by the acute consumption of the ginger extracts and the anti diabetic drug. Hence, the physiological state of the body is not altered by first day consumption of ginger extracts as far as glucose release into the blood from the gastrointestinal tract is involved.

Chronic effect

The four weeks extracts administration has reduced the FBG by 25% in the normal groups and in the diabetic groups it was reduced by 47%, 35% and 46% in the groups given raw, cooked extracts and drug respectively. The ascertains the hypoglycemic effect of ginger extract and the drug as earlier mentioned. In all the extracts and drug treated groups the peak postprandial blood glucose was observed at 90 minutes. While the rate of increase was slightly reduced in all the groups it was significantly higher in the NC group (Table 2). This shows that though the chronic extracts and drug consumption extended the peak postprandial blood glucose to 90 minutes the rate of increase was not significantly altered except in normal rats given cooked ginger.

Pancreatic amylase activity

Chronic ginger extracts and drug administration clearly modulated or altered the pancreatic amylase activity in all groups (Table 3). In the normal rats the enzyme activity was increased by 1.15% by the raw extract but was reduced by 25% by the cooked extract while in the diabetic groups raw cooked extracts and the drug reduced the enzyme activity by 17%, 36% and 49% respectively. This corroborates past research findings that aqueous ginger extracts increased the activity of amylase in the small intestine of normal rats though the percentage of increase was higher (10.7%) (Nwachuckwu and Ohiri 2012). There is scarcity of

reports on the effect in diabetic rats hence appreciable comparison could not be made.

CONCLUSION

Chronic administration of raw and cooked ginger extracts as well as the drug reduced the activity of pancreatic amylase in normal and diabetic rats (except in normal rats given raw ginger extract), hence, this modulation may be a possible mechanism of the hypoglycemic effect of the spice but the effect on the interfering absorption unit should be explored amongst other feasible mechanisms.

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