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Full Length Research Paper

Raw and Cooked Ginger (Zingiber Officinale Roscoe) Extracts Alter Pancreatic Amylase Activity in Normal and Steptozotocin-Induced Diabetic Rats

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ABSTRACT

Very many scientific findings have reported the hypoglycemic effect of different extracts of ginger,*Zingiber officinale*, few have delved into the effect of the raw extract and the possible mechanisms of action while very few have explained this effect of the cooked extract of the spice, the form in which it is most commonly consumed. Hence, this study aimed at determining the effect of raw and cooked ginger extracts on pancreatic amylase activity in normal and streptozotocin-induced diabetic rats. Seventy male Albino rats were divided into 7 groups and were treated thus: A- normal or negative control, B- normal rats given raw ginger extract; C- normal rats given cooked ginger extract; D- diabetic control; E- diabetic rats given raw ginger extract; F- diabetic rats given cooked ginger extract; and G- diabetic rats given glibenclamide. The extracts were administered as a single daily oral dosage of 4ml/kg body weight for 4 weeks after which the animals were sacrificed and the pancreas removed for pancreatic amylase activity assay. ANOVA and LSD were used to compare the mean of the data. Both extracts increased the enzyme activity in normal and diabetic rats. This increase, though lower in cooked extract, was still significant ($p < 0.05$). The acute and chronic effect of the extracts and after starch load followed a similar trend with the extracts prolonging the peak blood glucose level to 1.5 hours at the chronic state. Ginger extracts and the anti diabetic drug did not inhibit the activity of pancreatic amylase, hence, this is not a possible mechanism for the hypoglycemic effect of the spice though these effect a longer time for the blood glucose to reach its peak value after chronic administration.

Keywords: Herbal extracts, hypoglycemia, spice, blood glucose.

INTRODUCTION

Ginger is a food adjunct grown and used all over the world. The Roscoe type which is the white species of *Zingiber officinale* is more commonly grown and consumed than the Rubra which is the red type. Coupled with its use as spice ginger had also been reported to exert medicinal effect such as: anti diabetic (Mozaffari-Khosravi et al, 2014; Mahluji et al., 2013), hypolipidemic (Elshater et al, 2009; Al-Amin et al, 2006), anti-inflammatory (Ojewole, 2006) as well as in the treatment of nausea, constipation and other intestinal disorders (Baliga et al., 2012) and cancer prevention (Shukla and Singh, 2007).

Several studies have reported the hypoglycemic effect of different extracts and forms of the spice, few of these have explored the use of ginger juice while very few have identified the possible mechanisms of action. Sharma amd Shukla (1977) reported the hypoglycemic effect of ginger juice in normal and diabetic rats while Akhani et al., 2004 and Elshater et al., 009 reported similar effect of the juice in diabetic rats. Aqueous,

methanolic, ethanolic extracts as well as ginger powder and the 6-gingerol isolate were also observed to lower blood glucose significantly in drug-induced diabetic animals and type 2 diabetic patients (Mahliji et al., 2013; Sukalingam et al., 2013; Abdulrazaq et al., 2012, Jafri et al., 2011; Ojewole et al., 2006) but few scientific reports were made on the mechanisms of action of this effect.

The possible mechanisms of action of the anti diabetic effect of ginger may be : the modulation of antioxidant enzymes and inflammatory cytokines (Morakinyo et al,. 2011); activation of antioxidant enzymes thus preventing oxidative damage which exacerbate diabetic complications (Shanmugam et al.,2011); increased insulin secretion and insulin sensitivity (Iranloye et al., 2011); acting as anti glycating agents (Saraswat et al., 2010) and acting like insulin in vitro study (Broadhurst et al., 2000). All these were channeled towards prevention of diabetic complications but did not elucidate the effect of ginger on postprandial blood glucose as modulated by the digestive enzymes. In vitro study had reported the activation of amylase isolated from rats by ginger (Rao et al., 2003) while another similar but more recent study observed an inhibitory effect (Akinyemi et al, 2010). Chronic consumption of ginger-enriched diets was found to increase α-amylase activity in the small intestine but reduced it in the large intestine significantly in albino rats (Nwachuckwu and Ohiri, 2012).

The conflicting nature of these scientific findings as well as the abject scarcity of scientific reports on this effect about cooked ginger extract requires further research study on this concept. In view of this, this study examined the modulatory effect of raw and cooked ginger extracts on pancreatic amylase activity which may be a possible mechanism of action of the hypoglycemic activity of the spice.

MATERIALS AND METHOD

Preparation of extracts

Fresh ginger rhizomes (*Zingiber officinale* Roscoe) was purchased from Bodija market in Ibadan, Nigeria The method used by Elshater et al., 2009 was used to prepare the raw extract with slight modification. The spice was washed, weighed, peeled, weighed and wet-milled using plate attrition mill (Amuda Plate mill, India). This was then sieved using cheese cloth and 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 Thermo cool gas cooker, India. This was allowed to cool and stored in a plastic jar at 2^{o}C until use.

Collection of rats

Male albino rats (70) of weight range 140-170g were purchased from the Experimental Animals Unit of the Department of Veterinary Physiology, University of Ibadan, Ibadan, Nigeria. These were acclimatized for two weeks and were fed rats pellets and tap water ad libitum. These were grouped according to weight in seven plastic cages with 10 rats in each group. All animals were treated in accordance with the ethical approval (NHREC/05/01/2008a) obtained from the U.I/ U.C.H. Ethical Review Committee of the College of Medicine, University of Ibadan, Ibadan, Nigeria.

EXPERIMENTATION

Rat Grouping

The seven groups of rats were designated thus: A- normal control group, B- normal rats given raw ginger extract (4ml/kg body weight daily for 4 weeks), C- normal rats given cooked ginger extract, D- diabetic control group, E- diabetic rats given raw ginger extract, F- diabetic rats given cooked ginger extract and G- diabetic rats given glibenclamide (5mg/kg body weight), The control groups were given distilled water instead of ginger extract.

Induction of diabetes

Diabetes was induced by intra peritoneal injection of streptozotocin (Sigma Aldrich, Germany) at 60mg/kg body weight as used by Al-Amin et al, 2006 and fasting blood glucose (FBG) was monitored until stable hyperglycemia was confirmed using ACCUCHEK Active Glucometer, Roche, Germany.

Acute and chronic effect

This was determined following the method of Tomo et al., 2004 with slight modification. Blood glucose of overnight fasted rats was recorded after which starch and extracts were given at 2g/kg body weight and 4ml/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.

Pancreatic amylase activity assay

After 4 weeks of extracts administration, the animals were sacrificed by cervical dislocation and the pancreas removed washed with Phosphate Buffered Saline (PBS-pH 7.2) and 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 mortar and pestle. This was then centrifuged at 10,000rpm for15 minutes at 4^{o}C 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 37^{o}C 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):

$$\text{Amylase activity (U/L)} = \frac{\text{ODsample} - \text{ODblank}}{\text{ODcalibrator} - \text{ODwater}} \times 550$$

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 ($p < 0.05$).

RESULTS AND DISCUSSION

Acute effect

Pancreatic amylase is an enzyme involved in the digestion of carbohydrates in the small intestine thus contributing to the hepatic blood glucose via absorption from the small intestine. After the starch and extract load immediately after diabetes was induced, the blood glucose reached the peak value at 1 hr in almost all the groups. 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 isolated from white kidney beans *(Phaseolus vulgaris*) but in the negative control group the peak value, though higher than the experimental group, was observed at 50 minutes. In the negative control the blood glucose increased by 16 and 10% at 30 and 60 minutes but reduced by 5.2, 6.9, 5.0 and 3.4% consequently as can be deduced from Table 1.

Variation in BG was noticed in B with 10 and 10% increase at 30 and 60 minutes coupled with the 6.8, 8.2, 8.8 and 8.0% reduction at 90, 120, 150 and 180 minutes respectively. In the diabetic control group (D), a high increase (13.9%) was observed at 60 minutes postprandial but slowly reduced afterwards. In Groups E, F and G the increase in BG at 30 and 60 minutes was about 4.0% while the disappearing rate after 60 minutes was higher than that of diabetic control though this was not significant ($p<0.05$).

Chronic effect

The major noticeable observation in the chronic effect is that the peak value of BG in the groups treated with extracts and drug have shifted from 60 minutes to 90 minutes (Table 2). There was a marked positive difference ($p<0.05$) between the increasing rates of BG before 90 minutes and after this time in both normal and diabetic groups treated with ginger extracts and drug respectively. This may be as a result of increase in the digestion, absorption and utilization of glucose by the body cells which is a reflection of changes in various biochemical parameters involved in glucose metabolism. In the negative control group the BG significantly increased ($p< 0.05$) more than the increase observed in the acute effect and the increase in the disappearing rate of BG after 60 minutes was not significant ($p<0.05$. In the diabetic control both the increasing and disappearing rates of BG before and after 60 minutes respectively were significantly lower than in the acute effect thus reflecting the inhibition of biochemical parameters involved in anabolism and catabolism.

In overall, the increase in FBG at the first day ginger administration (acute effect) observed from 0 minute to 60 minutes in A,B,C,D,E,F was 30.60, 33.40, 29.00, 40.33, 31.00, 33,67 and 27.34 respectively while after 4 weeks (chronic effect the FBG) the increase observed from 0 to 90 minutes in B,C,E, F. G H was 37.10, 40.20, 32.33, 29.00 and 28.00mg/dl but in A and B this was 36.40 and 36.33 respectively with the peak values observed at 60 minutes.

Pancreatic Amylase activity

Four weeks of ginger extracts and drug administration resulted in a significant increase ($p< 0.05$) in pancreatic amylase activity in both normal and diabetic rats (Table 3). This must have resulted into the marked increase in blood glucose after starch load in the chronic effect of the extracts (Table 2) compared to the acute effect. Another factor that may be responsible for this is the absorption of glucose into the blood. The enzyme activity was highest in both normal and diabetic rats given raw ginger extract though the values were significantly different in these two groups ($p< 0.05$). .Raw and cooked ginger extracts increased the enzyme activity by 28 and 7% respectively in normal rats while in diabetic rats the increase was 50, 31 and 32% by raw extract, cooked extract and drug respectively as can be deduced from Table 3.

Nwachuckwu and Ohiri in 2012 also reported an increase in the amylase activity in the small intestine from 238.00 U/L in the control group to 263.50 U/L in the albino rats fed diet 2.5% ginger for 8 weeks but the amylase activity in the large intestine was significantly reduced. The increase in pancreatic amylase activity by was also reported by Rao et al., 2003 on the other hand, Akinyemi et al.,2010 reported an inhibitory effect of aqueous ginger extract on the enzyme. Even though the effect on the amylase activity in the small intestine as reported by Nwachuckwu and Ohiri in 2012 followed the same trend with the observation of this study, the exists disparity in the values obtained. This may be due to differences in the sites of the enzyme as well as in the duration of ginger administration.

Table1. Acute effect of ginger, extracts on blood glucose (mg/dl) after starch load

Groups	BG/SD 0min	BG/SD 30 min	BG/SD 60 min	BG/SD 90 min	BG/SD 120 min	BG/SD 150 min	BG/SD 180 min
A	110.90/5.5	128.80/7.2	141.50/5.4	134.10/6.6	124.80/6.5	118.50/3.3	114.30/3.7
B	110.90/6.7	128.00/7.2	144.30/5.4	134.40/6.6	123.40/6.5	112.80/7.3	103.70/5.3
C	114.00/3.5	129.00/5.5	143.00/6.3	127.20/4.6	121.40/3.2	115.20/6.5	112.00/4.5
D	379.00/4.6	393.00/5.3	419.33/5.9	402.33/9.6	395.67/7.1	386.00/5.3	376.33/6.0
E	373.67/7.6	389.00/6.9	404.67/9.8	395.67/13.3	385.00/8.2	373.33/8.6	375.00/4.4
F	381.33/4.5	394.00/5.0	415.00/5.3	394.67/3.8	384.33/4.0	379.69/1.2	371.67/1.5
G	374.33/8.4	390.64/7.6	400.00/6.6	401.67/18.2	386.33/12.5	377.67/12.1	372.61/5.1

BG/SD- Blood glucose ± standard deviation

Table2. Chronic effect (4 weeks) of extracts on blood glucose (mg/dl) after starch load

Groups	BG/SD 0min	BG/SD 30min	BG/SD 60min	BG/SD 90min	BG/SD 120min	BG/SD 150min	BG/SD 180min
A	110.30/5.6	136.70/4.5	146.70/5.6	136.80/6.8	125.70/4.4	118.40/3.4	112.50/6.0
B	82.50/2.1	97.30/6.9	109.70/5.3	119.60/3.4	112.90/8.8	102.60/8.9	93.10/7.4
C	85.00/3.2	100.40/5.6	113.00/8.8	125.2/4.0	111.80/7.5	102.40/7.8	91.60/8.8
D	431.00/3.6	452.00/7.6	467.33/4.5	459.00/4.4	450.33/2.1	447.67/3.5	440.33/9.5
E	114.67/2.5	127.67/1.5	137.33/2.9	147.00/1.7	130.67/1.5	124.00/2.7	115.67/2.1
F	115.33/1.2	128.67/0.6	138.33/1.2	144.33/4.0	135.67/4.7	125.57/4.7	114.33/4.9
G	115.00/2.0	130.67/4.6	142.33/5.7	143.00/9.6	131.33/3.8	123.33/4.4	114.67/3.5

BG/SD- Blood glucose ± standard deviation

Table3. Effect of ginger extracts on pancreatic amylase activity in normal and diabetic rats

Group	Amylase activity (U/L)
A	423.97 ± 5.13
B	543.90 ± 2.44
C	454.59 ± 3.14
D	356.10 ± 2.45
E	532.37 ± 2.17
F	466.29 ± 1.76
G	469.26 ± 0.65

CONCLUSION AND RECOMMENDATION

Both raw and cooked ginger extracts increased pancreatic amylase activity in normal and diabetic rats but the raw form increased it more than the cooked form. The effect of the drug was similar to that of the cooked extract in diabetic rats. This effect was reflected in the chronic effect of the extracts on blood glucose after starch load by the marked increase in the hepatic blood glucose which was significantly lower in the acute effect. It can thus be deduced that inhibition of pancreatic amylase is not a possible mechanism for the hypoglycemic effect of both ginger and the drug hence other feasible channels need to be explored among which is the effect on glucose absorption. More still, the increasing disappearing rate of glucose from the blood after 90 minutes (chronic effect) denotes increased uptake and utilization of glucose by the body cells which also needs to be ascertained.

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