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Effects of Aqueous Extract of Spices Mixture Containing Curry, Garlic and Ginger on Plasma Glucose and Lipids in Alloxan-induced Diabetic Rats

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Abstract: With increasing use of spices worldwide in the face of increasing burden of Diabetes Mellitus (DM), especially type 2, coupled with claims about the favourable effects of spices on some health conditions, the present study investigated the effect of aqueous extract of spices mixture containing curry, garlic and ginger on plasma glucose and lipids in alloxan induced diabetic rats, a type 2 DM model. The animals were assigned into six groups (I-VI) of six animals per group. Group I-III animals were made diabetic by intraperitoneal administration of alloxan while group IV-VI were non diabetic. Groups I & II and V & VI were administered 300 mg and 600 mg/Kg body weight respectively, of the extract by oral compulsion for four weeks while groups III and IV acted as diabetic and non-diabetic control respectively. Plasma glucose and lipid profile were analyzed by standard laboratory methods. The extract had no significant effect on body weights of the animals irrespective of their diabetic status. However, the extract had significant ($p<0.05$) hypoglycaemic effect on both diabetic and non-diabetic rats, with plasma glucose lower in the groups (diabetic and non-diabetic) treated with 600 mg/kg body weight of the extract in comparison to those treated with 300 mg/kg body weight (4.43±0.56 vs. 5.03±0.50 and 4.08±0.13 vs. 4.41±0.22 mmol/l respectively). While plasma High Density Lipoprotein-Cholesterol (HDL-C) was comparable among the animal groups, plasma Total Cholesterol (TC), Triglyceride (Tg) and Low Density Lipoprotein-Cholesterol (LDL-C) were significantly ($p<0.05$) lower in the groups (diabetic and non-diabetic alike) treated with the extract when compared with those untreated. This effect however appeared to be abolished at higher concentration of the extract as evidenced by lower decreases in the lipid fractions at the concentration of 600 mg/kg body weight against that at 300 mg/kg body weight. In conclusion, intakes of curry, garlic and ginger concurrently at culinary dose exerts beneficial effects on plasma glucose and lipids in health and disease. It also reaffirms the safety of spices combinations as practiced currently.

Key words: Spices mixture, type 2 diabetes model, hypoglycaemia, hypolipidaemia

INTRODUCTION

The burden of diabetes mellitus type 2, characterized by insulin resistance and hyperglycaemia is on the increase worldwide (Razieh *et al*., 2007). The disease has been recognized as an important public health problem in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive and unaffordable (Djrolo *et al*., 1998). Again, the incidence of cardiovascular diseases has been found to increase two-to-fourfold in people with type 2 diabetes mellitus (Raza and Movahed, 2003). The inability of the modern therapy to control all the pathophysiological aspects of diabetes and its complications coupled with the enormous costs it poses on the economy of the developing nations of the World, underscore the alternative strategies urgently sought (WHO, 2002). Plants used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the management of this disease. Amongst such plants reported to have beneficial effects in the treatment of diabetes are spices such as cinnamon, cloves, bay leaves, ginger, turmeric, garlic amongst others (Khan *et al*., 2003; Broadhurst *et al*., 2000; Liu *et al*., 2007; Srinivassan, 2005), although both experimental and epidemiological studies have been consistently equivocal on their antidiabetic effects. While some studies have reported beneficial effects of these plants on some biomarkers of the disease, such as plasma glucose, lipid profile and antioxidant capacity (Ojiako and Nwanjo, 2009; Ugwuja *et al*., 2008; Al-Amin *et al*., 2006), others did not (Islam and Choi, 2008). Also most studies on medicinal/culinary plants have focused on individual plant. However, spices are seldom consumed singly, but rather as mixtures of two or more spices used to improve flavour of foods. In the light of previous findings of beneficial effects of dietary supplement containing

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mixture of raw curry, garlic and ginger on plasma glucose and lipids on normal rats, this study is aimed at investigating the effects of aqueous extract of raw spices mixture containing curry, garlic and ginger on alloxan-induced diabetic rats with specific interest on plasma glucose and lipid profile. Since the use of spices is on the increase, the result from this study would provide scientific evidence of the safety or otherwise of their uses by individuals with or at risk of diabetics mellitus.

MATERIALS AND METHODS

Animals: Male Wister albino rats (n = 36), weighing 108-162 g purchased from animal house of the Department of Pharmacy, University of Nigeria, Nsukka were randomly assigned into six (6) groups (I-VI) of six (6) rats per group. The animals were allowed free access to feed and water *ad libitu* for a period of one week to allow them acclimatise. All the rats received human care in accordance with the National Institute of Health guidelines for the care and use of laboratory animals.

Induction of diabetes: Diabetes mellitus was induced in rats in groups I-III by intraperitoneal injection of 200 mg/Kg body weight of alloxan dissolved in distilled water while groups IV-VI were not induced. Fasting blood glucose levels were determined after three days of alloxan injection with a glucometer (ACCUTREND GC, Boerhinger, Mannheim, Germany), using blood from tail tips and diabetes mellitus was confirmed by elevated fasting plasma glucose > 7.8 mmol/l.

Preparation of spices' mixture: Raw spices powder of curry, garlic and ginger were purchased from Abakpa main market in Abakaliki metropolis. The spices were mixed together in equal ratio to form a uniform powder. This was soaked in 400 ml of distilled water overnight with occasional shaking after which it was filtered. The filtrate was dried on a rotary evaporator to give a semi-solid residue from which appropriate weight was measured and dissolved in 2.0 ml of physiological saline to obtain the desired concentration of 300 mg/ml of the extract.

Treatment of animals: Animals in groups I & II and groups V & VI received 300 mg and 600 mg/kg body weight of the extract respectively while animals in groups III and IV were given 2.0 ml of physiological saline daily and served as diabetic and non diabetic controls respectively. Throughout the duration of the study, which lasted for four (4) weeks, all the animal were maintained on normal rat feed. At the end of the experiment, the animals were fasted for 12 h after which they were sacrificed and blood (6.0 ml) collected and dispensed into fluoride oxalate bottle (2.0 ml) and EDTA bottles (4.0 ml) for the estimation of plasma glucose and lipids respectively.

Biochemical analyses: Plasma was isolated from the blood samples by centrifugation at 2000 g for 5 min in a laboratory centrifuge. Plasma glucose was determined by glucose oxidase method as described by Barham and Trinder (1972), plasma total cholesterol was estimated using the method described by Lopez-Vitrella *et al.* (1977), HDL-cholesterol and LDL-cholesterol were determined as described by Lopez-Vitrella *et al.* (1977) and Assmann *et al.* (1984) respectively.

Statistical analysis: All the data were analyzed for mean and standard deviation. Comparison of variables among groups was done using one way Analysis of Variance (ANOVA) and value is considered statistically different when p value is less than 0.05.

RESULTS

From Table 1, all the animals showed increments in their body weights irrespective of their diabetic status, although there was no significant difference in their final body weights.

Table 2 shows that aqueous extract of spices mixture of curry, garlic and ginger had significant ($p<0.05$) hypoglycaemic effect on both diabetic and non-diabetic rats, with the effect higher at higher concentration of the extract, as the plasma glucose was lower in the animal groups (diabetic and non-diabetic) treated with 600 mg/kg body weight of the extract in comparison to those treated with 300 mg/kg body weight of the extract (4.43±0.56 vs. 5.03±0.50 and 4.08±0.13 vs. 4.41±0.22 mmol/l respectively). However, while plasma High Density Lipoprotein-Cholesterol (HDL-C) was comparable among the animal groups, plasma Total Cholesterol (TC), Triglyceride (Tg) and Low Density

Table 1: Effect of aqueous extract of spices mixture containing curry, garlic and ginger on body weight of alloxan-induced diabetic rats[1,2]

	Diabetic case		Non-diabetic case		Controls	
Duration/day	Group I (300 mg/kg body weight)	Group II (600 mg/kg body weight)	Group V (300 mg/kg body weight)	Group VI (600 mg/kg body weight)	Group III (Diabetic)	Group IV (Non-diabetic)
0	79.6±5.6	81.2±6.1	81.6±6.7	81.1±4.5	77.9±2.8	80.8±3.3
7	84.6±4.2	82.6±5.1	83.7±4.9	83.7±7.4	79.1±3.2	83.7±3.6
14	85.3±3.0	85.7±2.9	89.3±7.4	87.6±5.7	82.3±2.9	88.9±4.2
21	87.3±3.8	86.6±4.1	90.7±7.4	93.9±4.8	88.6±4.5	92.7±4.4
28	95.4±4.6	94.3±3.9	99.4±6.8	96.6±4.8	94.6±3.7	97.4±2.9

[1]Values are expressed as mean ± standard deviation. [2]Weight of the animals were expressed in grams (g)

Table 2: Effect of aqueous extract of spices mixture containing curry, garlic and ginger on plasma glucose and lipids of alloxan-induced diabetic rats[1,2]

	Diabetic case		Non-diabetic case		Controls	
Plasma parameters	Group I (300 mg/kg body weight)	Group II (600 mg/kg body weight)	Group V (300 mg/kg body weight)	Group VI (600 mg/kg body weight)	Group III (Diabetic)	Group IV (Non-diabetic)
Glucose (mmol/l)	5.03±0.50[a]	4.43±0.56[a]	4.41±0.22[a]	4.08±0.13[a]	8.02±0.63	5.67±1.23
TC (mmol/l)	4.75±1.50[b]	4.98±1.87[b]	3.78±0.81[b]	3.80±0.71[b]	5.75±2.47	4.12±0.98
Tg (mmol/l)	1.40±0.57[c]	1.73±1.28[c]	1.29±0.19[c]	1.33±0.67[c]	2.35±1.21	1.52±2.01
LDL-C (mmol/l)	3.08±0.78[d]	3.28±1.37[d]	3.15±0.70[d]	3.21±0.15[d]	3.76±1.40	3.54±0.97
HDL-C (mmol/l)	1.03±0.85	1.05±0.66	1.01±0.51	0.83±0.50	1.00±0.68	0.97±0.63

TC: Total Cholesterol; Tg: Triglyceride; LDL-C: Low Density Lipoprotein-Cholesterol; HDL-C: High Density Lipoprotein-Cholesterol.
[1]Values are expressed as mean ± Standard deviation.
[2]Values with the same superscript along the row are significantly ($p<0.05$) different from the diabetic control

Lipoprotein-Cholesterol (LDL-C) were significantly ($p<0.05$) lower in the animals (diabetic and non-diabetic alike) treated with the extract when compared with those untreated. This effect however appeared to be abolished at higher concentration of the extract as evidenced by lower decreases in the lipid fractions at extract concentration of 600 mg/kg body weight against that at 300 mg/kg body weight.

DISCUSSION

Data from the present study show that aqueous extract of spices mixture containing curry, garlic and ginger exert hypoglycaemic and hypolipidaemic effects, but while hypoglycaemic effect was dose dependent, the hypolipidaemic effect tended to be abolished at higher concentration. Studies that investigated effects of spice mixture are scarce. For example, spice mixture of cumin, coriander and red pepper has been reported to affect bacteria metabolism in the serum (Sugawara and Suzuki, 1997). Also, *Amrita Bindu*-a salt-spice-herbal mixture has been found to possess antioxidant potential against free radical-induced oxidative damage (Ntarajan *et al*., 2006). Previously, we have reported both hypoglycaemic and hypolipidaemic effects of spices mixture made of equal proportions of curry, garlic and ginger on normal rats at 2% w/w, a concentration beyond which there were significant increases in both mean plasma glucose and total cholesterol (Ugwuja *et al*., 2008), which is consistent with the findings of the present study. Cinnamon, garlic and ginger has been reported to exert both hypoglycaemic and hypolipidaemic effect individually (Khan *et al*., 2003; Thompson *et al*., 2006; Gorinstein *et al*., 2006; Al-Amin *et al*., 2006). However, one study (Islam and Choi, 2008) has specifically reported that aqueous extract of ginger and garlic lacks effect on plasma lipids but are insulinotropic rather than hypoglycaemic in type 2 diabetes model, with ginger having better antidiabetic effect than garlic. Also, garlic and ginger has been found to provide some help for persons with hyperlipidaemia (Ali *et al*., 2000; Chetty *et al*., 2003). Although the mechanism by which spices such as curry, garlic and ginger lower plasma glucose or/and lipids has not been fully elucidated, laboratory data has proved that garlic for example, contain many biologically and pharmacologically important fat soluble organosulfur compounds such as Diallyl Sulphide (DAS), Diallyldisulfide (DADS), Diallyl Trisulfide (DATS) as well as water soluble S-allylcysteine which are beneficial to individuals suffering from cardiovascular, neoplastic and several other diseases (Agarwal, 1996; Alpers, 2009). Nevertheless, two mechanisms have been suggested by which garlic for instance lowers plasma cholesterol. These include inhibition of cholesterol and fatty acid synthesis (Ojiako and Nwanjo, 2009; Ojiako and Nwanjo, 2006). For instance, garlic and garlic-derived organosulfur compounds, including S-allylcysteine have been found to inhibit 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA), a rate limiting enzymes in the biosynthesis of cholesterol (Liu and Yeh, 2002). Also HMG-CoA activity has been reported to be significantly reduced in rat microsomes after garlic consumption (Merat and Fallahzadeh, 1996). Additionally, Inhibition of fatty acid synthesis by reduction in the incorporation of C-acetate into fatty acid has been demonstrated (Ojiako and Nwanjo, 2006). Again, dietary garlic has been reported to inhibit the synthesis of lipids in the liver and increases the levels of serum insulin, thereby increasing the glycogen in the liver and lowering serum glucose (Chang and Johnson, 1980). The reason for the loss of hypolipidaemic actions by the aqueous extract at higher concentration is obscure. However, a whole family of sulphur compounds (terpenoids, phenolic, allicin) known as sulphinyl disulphides in curry, garlic and ginger are known to undergo exchange reaction with SH-groups of enzymes and other proteins in the body spontaneously at physiological pH and temperature and may have inhibitory action on their activities (Rabinkov *et al*., 2000). It is therefore suggested that at higher concentration of the aqueous extract of the spices mixture, these chemical interactions might be strong enough to produce metabolic alterations which is manifested by abolition of the hypolipidaemia. Arguably, toxic effect of this extract at higher concentration can not be said to be responsible for the abolition of hypolipidaemic effect observed in the present study, as the glucose-lowering effect was

enhanced at the same concentration at which hypolipidaemic action seemed to be abolished. Regrettably, neither the liver enzymes nor histological examination was carried out on our subjects to rule out this possibility. However, it has been previously reported that garlic has a hypolipidaemic effect at lower dose, but at higher dose produces hypoglycaemic effect (Thompson *et al*., 2006). In corroboration with earlier study (Ugwuja *et al*., 2008), this study also showed that aqueous extract of spices mixture had no significant effect on body weight of both the diabetic and non-diabetic rats. Study on aqueous extracts of herbs has shown similar results (Proph *et al*., 2006). We therefore conclude that concurrent intake of curry, garlic and ginger at culinary dose in the diet has beneficial effect on plasma glucose and lipids in health and disease and reaffirms the safely of spices combinations as practiced currently.

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