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Effects of Clove and Fermented Ginger on Blood Glucose, Leptin, Insulin and Insulin Receptor Levels in High Fat Diet-Induced Type 2 Diabetic Rabbits

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Summary: The aimed of this research is to evaluate the effects of clove and fermented ginger supplements on blood glucose, serum insulin, insulin receptor and Leptin levels of high fat diet-induced type 2 diabetes mellitus in rabbits. Clove and ginger are spices with records of medicinal value over decades. Thirty males rabbits weighing, 1–1.5kg were used for the research. Type 2 diabetes was induced by feeding the animals with a high fat diet for a period of eight weeks. Blood glucose levels were determined after the induction period and rabbits having 140 mg/dL and above were selected for the study. The animals were grouped into six groups with five (n=5) rabbits in each group: Group 1 (Normoglycemic control group.) received normal feed and distilled water *ad libitum* for six weeks; Group 2 (Diabetic negative control group.) received normal feed and distilled water *ad libitum* for six weeks; Groups 3 (Diabetic positive control.) received cholestran 0.26g/kg and normal feed for a period of six weeks; Group 4 and 5 (diabetic rabbits) were fed on 12.5%, clove and 12.5% fermented ginger respectively for a period of six weeks; while Group 6 were co-fed on 12.5% clove and 12.5% fermented ginger for a period of six weeks. Fasting blood glucose levels were determined at weekly interval during the treatment period. At the end of the experiment, the rabbits were euthanized by cervical dislocation and blood samples were collected for the determination of insulin, insulin receptor and leptin levels. A significantly ($P<0.05$) decrease in blood glucose levels was recorded in the supplements treated groups compared to diabetic control group. Clove supplement been most effective and sustaining in antihyperglycemic activity, also appears with a significant decreasing effect on leptin levels compared to diabetic control group. A significant increase in insulin levels was also noted in the fermented ginger treated group along with higher levels of Leptin compared as compared to control group. In conclusion the result of the study show that clove and fermented ginger supplementation possesses anti-diabetic properties and may help in the control of hyperleptinaemia in type 2 diabetes.

Keywords*:* Clove, Ginger, Type 2 Diabetes, Leptin and Insulin.

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INTRODUCTION

Consumption of high Fat Diet (HFD) or western diet has been adopted in many population across the globe, which is associated with the largest incidence of metabolic syndrome in the world (Buettner *et al*., 2007). Chronic intake of HFD leads to obesity, defective insulin secretion or function which results to various metabolic aberrations and diabetes mellitus (Jimoh *et al*., 2015). The associated impairments include hyperglycaemia due to defective insulin-stimulated glucose uptake; up- regulated hepatic glucose production and dyslipidaemia (Baxter and Webb, 2009).

Excessive short term consumption of high fat diet has been associated with an increased incidence of enhanced oxidative stress, consequently, leading to high levels of circulating free fatty acids (FFA) and glucose which are potent inducers of reactive oxygen species (ROS) formation in cells (Supale *et al*., 2012). Lipotoxicity impairs cell function and viability due to chronic exposure to FFA, leading to the induction of β- cell endoplasmic reticulum stress, and glucose-induced β-cell dysfunction and apoptosis (Tang *et al*., 2013). The excessive consumption of high fat diet has been associated with an increased incidence of type2 diabetes mellitus (T2 DM) through insulin resistance associated with hyperleptinaemia (Keaney *et al*., 2003; Demarco *et al*., 2010; Otani, 2011).

Type 2 diabetes mellitus is now a common, growing, serious and costly, but potentially preventable disease appearing across populations globally (Adams, 2011). Continuous consumption of calories-rich meals, junk food and sedentary lifestyle has culminated into an epidemic of diabetes worldwide. The existing management of diabetes is indeed costly and associated with many side effects ranging from constipation, abdominal discomfort to hypoglycemia.

Hence, the need for an effective alternative therapy with fewer or minimum side effects.

Clove buds and ginger rhizome are important spices used across the world as natural remedy and in folklore for many disease management (Bode and Dong, 2011). Positive therapeutic effects of their individual extracts have been studied in type1 diabetic animal model with paucity in some results (Alnoory *et al*., 2013). This study aimed to determine the effects of Clove (*Syzygium aromaticum*) and Fermented Ginger (*Zingiber officinale*) Supplements on Blood Glucose, leptin, Insulin and Insulin Receptor Levels in High Fat Diet- Induced Type 2 Diabetes in Rabbits.

MATERIALS AND METHODS

Collection of Plant material.

Clove (*Syzygium aromaticum*) buds and Ginger (*Zingiber officinale*) rhizome were purchased at Tudun-Wada Market Zaria, Kaduna Nigeria. They were authenticated at the herbarium unit of the Department of Biological Sciences, Faculty of life Sciences, Ahmadu Bello University, Zaria, with voucher numbers 900127 and 2261 respectively.

Animals

A total of thirty (30) male rabbits (New Zealand) aged 5-8 weeks were used for the study, animals were housed in the Animal house of Department of Human Physiology, Faculty of Medicine Ahmadu Bello University Zaria, under standard laboratory conditions and had access to feed (Pellet Growers feed) and water *ad libitum.* Animal care and use was in accordance with the guide for the care and use of laboratory Animals, institute for Laboratory Animal Research, National Institute of Health (NIH Publication No.80-23; 1996).

Drugs /Reagents used

All chemicals used were of analytical gradesi. Cholesterol (powder) was purchased from KEM LIGHT Laboratory PVT. LTD, Mumbai India. (CAS: 57-88-5, m.w: 386.67, lot no.: 100814). Cholestran powder (made in Egypt PHARCO pharmaceuticals Alexandria was purchased from Amira Pharmacy, Tudun wada Zaria. Methylated spirit, cotton wool. Rabbit INS ELISA Kit (No.GA-E0015RB) LOT 20151012, REF E20151012001; v. Rabbit ISR ELISA Kit (No.GA-E0016RB) LOT 20151012, REF E20151012002, and LEP ELISA Kit (No.GA-E0017RB) LOT 20151012, REF E20151012003, GenAsia Biotech Co., Ltd., 7th floor,Wujiaochang Technology Building, No. 1675, Huangxing Road, Yangpu District, Shanghai, China. Digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany). Glucose- test strips for assessment of plasma glucose levels, manufacture by accu-check Advantage II, Roche Diagnostics GmbH Germany.

Experimental Induction and Determination of Diabetes Mellitus

The rabbits were handled in accordance with the principles guiding the use and handling of experimental animals, ABU, Zaria. Ethical approval was obtained, with Approval No: ABUCAUC/2017/048. The animals were fasted from feeds for 12-14 hours prior to commencement of the experiment, but allowed water *ad libitum*. Type 2 diabetes mellitus was induced by feeding the animals with a high fat diet (2% cholesterol, 20% groundnut mill and 10% groundnut oil) as reported by Jimoh *et al*. (2015) for a period of eight weeks. Fasting blood glucose levels were determined by using the glucose oxidase method (Trinder, 1969) Rabbits having glucose levels greater than 140 mg/dl were considered Hyperglycemic. The results were reported as mg/dl (Rheney and Kirk, 2000).

Experimental Design

After the induction of type 2 diabetes mellitus, rabbits having fasting blood glucose levels of 140mg/dL (Jimoh *et al*., 2015) and above were selected for the study. The animals were randomly assigned into experimental and control group of five (5) rabbits each, as follows;

Group i: Normal rabbits fed on animal standard feed

Group ii: Diabetic rabbits fed on standard feed for six weeks

Group iii: Diabetic rabbits fed on standard feed and administered cholestran (0.26g/kg body weight) for six weeks.

Group iv: Diabetic rabbits fed on clove 12.5% supplement for six weeks.

Group v: Diabetic rabbits fed on 12.5% fermented ginger supplement for six weeks.

Group vi: Diabetic rabbits fed on clove 12.5% + fermented ginger 12.5% supplements for six weeks.

Blood Sample Collection and Serum Preparation

At the end of the six weeks treatment period, the rabbits were euthanized by cervical dislocation and blood samples were collected from the animals through cardiac puncture. About 5 mL of blood were collected into specimen bottles and allowed to clot and separated by centrifugation at 3,000 g for 10 minutes using Centrifuge Hitachi (Universal 32). The supernatant obtained were used for the determination of insulin, and leptin concentrations.

Biochemical Estimations

The sera were used for the determination of serum insulin and leptin levels, using rabbit ultra-sensitive enzyme-linked immunosorbent assay (ELISA) kits (GenAsia Biotech, Co., Ltd. Shanghai, China), with catalogue numbers, GA-E0015RB (insulin) and GA-E0017RB (leptin) according to the manufacturer's instructions. The principles were based on biotin

double antibody sandwich technology (Schmidt *et al*., 2012). Serum insulin receptor was estimated using rabbit ISR (insulin receptor) ELISA kit.

Statistical Analysis

Data obtained from the study were expressed as mean ± SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA), followed by Tukey's post hoc test. Values of $P \leq 0.05$ were considered as significant (Duncan *et al*., 1977).

RESULTS

The results of blood glucose level (Table 1) revealed a significant ($P < 0.05$) decrease in blood glucose level at the second and fourth week of treatment in the diabetic model rabbits groups treated with clove 12.5%, fermented ginger 12.5%, and the combined clove 12.5% + fermented ginger 12.5% supplements, compared to diabetic model rabbits group fed on standard feed. While at the fifth week only, the group on clove 12.5% supplement showed a significant decrease in blood glucose level compared to that of diabetic model rabbits group fed on standard feed ($P < 0.05$).

Table 2) revealed a statistically significant ($P < 0.05$) increase in serum insulin level of all the HFD induced diabetic animal groups (groups 2-6), compared to the normal control group (group1). Moreover, a significant ($P < 0.05$) increase in group 5 (clove 12.5% supplement treated group), compared to serum level of insulin in diabetic rabbits fed on standard feed (group 2) was also recorded. Serum leptin level also showed a significant decrease in clove 12.5% supplement treated group (group 4) compared to the level seen in group 2 ($P < 0.05$). While a significant ($P < 0.05$) increase in serum insulin receptor was also seen in all HFD induced diabetic animals, compared to that of NC group.

Table 2: Effects of Clove, Fermented Ginger and co-administration of Clove and Fermented Ginger Supplements on Insulin, Leptin and Insulin-receptor in High Fat Diet Induced- type 2 Diabetes in Rabbits

Experimental Groups	Insulin (ng/ml)	Leptin (ng/ml)	Insulin Receptor (ng/ml)
Normal Control (NC)	9.00 ± 0.58	12.50 ± 1.44	15.00 ± 5.51
Diabetic Control (DC)	26.00 ± 2.51[a]	14.60 ± 1.96	29.40 ± 4.12 [a]
Diabetic + Cholestran (0.26g/kg)	25.60 ± 9.44[a]	12.60 ± 1.18	32.20 ± 4.05 [a]
Diabetic + Clove (12.5%)	31.20 ± 3.56 [a]	10.00 ± 1.60[b]	30.40 ± 3.64 [a]
Diabetic + Fermented ginger (12.5%)	37.20 ± 3.44[a,b]	14.20 ± 0.86	27.80 ± 2.58 [a]
Diabetic + Clove (12.5%) + Fermented ginger(12.5%)	26.40 ± 6.74 [a]	12.70 ± 2.63	28.60 ± 5.53 [a]

Superscripts: [a] = significant different compared to NC group; and [b] = significant difference when compared to DC group.

DISCUSSION

The observed decrease in blood glucose in the rabbit fed 12.5% clove and/or 12.5% fermented ginger could be explained by the effects of certain active constituents such as dehydrodieugenol and dehydrodieugenol B in clove, as well as Gingerol and shogaol present in ginger supplements which affect different level of insulin signaling cascade pathway or post insulin-receptor complex by increasing expression of glucose transporters and other molecular modulators of insulin activity resulting in increased glucose intake by muscle cells. (Arablou, *et al*., 2014; Hyun, *et al*., 2014).. Another mechanism of action of

Table 1: Effects of Clove 12.5%, Fermented Ginger 12.5% and co-administration of Clove12.5 and Fermented Ginger 12.5% Supplements on Blood Glucose Level in High Fat Diet Induced- type 2 Diabetes in Rabbits

Groups	BGL Week 0 (mg/dL)	BGL Week 1 (mg/dL)	BGL Week 2 (mg/dL)	BGL Week 3 (mg/dL)	BGL Week 4 (mg/dL)	BGL Week 5 (mg/dL)
Normal Control (NC)	109.80 ± 3.14	103.20 ± 4.62	101.80 ± 2.71	108.00 ± 1.92	95.20 ± 3.95	97.60 ± 2.04
Diabetic Control (DC)	140.00 ± 0.84[a]	119.00 ± 2.63[a]	123.40 ± 2.46[a]	140.80 ± 1.59[a]	125.40 ± 2.94[a]	114.00 ± 2.24[a]
Diabetic + Cholestran (0.26g/kg)	140.80 ± 1.53[a]	155.40 ± 2.87[a,b]	140.80 ± 0.86[a,b]	159.60 ± 2.84[a,b]	122.60 ± 0.98[a]	126.40 ± 1.17[a,b]
Diabetic + Clove (12.5%)	149.40 ± 2.04[a]	117.20 ± 1.59[a,]	128.00 ± 0.71[a,b]	162.60 ± 2.20[a,b]	104.60 ± 1.94[a,b]	105.20 ± 0.58[b]
Diabetic + Fermented ginger (12.5%)	149.20 ± 1.50[a]	132.80 ± 3.57[a,b]	134.00 ± 2.30[a,b]	141.80 ± 2.48[a]	112.20 ± 1.36[a,b]	132.00 ± 2.68[a,b]
Diabetic + Clove (12.5%) + Fermented ginger (12.5%)	149.40 ± 2.62[a]	143.20 ± 3.32[a,b]	136.60 ± 1.36[a,b]	168.00 ± 2.55[a,b]	112.20 ± 2.31[a,b]	127.80 ± 2.06[a,b]

Values are presented as mean ± SEM; n=5, P< 0.05 = significant. Values with superscripts: [a] = significant different in comparison to NC group, and [b] = significant different when compared to DC group.

ginger that may explain this action could be from inhibition of oxidative stress. Gingerol and shogaol are active ingredients present in ginger and may have contributed to the hypoglycemic effect observed in this study.). The findings of the present study on blood glucose level are similar to the work of Nafiseh*et al*. (2015), in which it was demonstrated that treatment with ginger significantly decreased blood glucose and other parameters tested in diabetic patients. Similarly, Mozaffari-Khosravi *et al*. (2014) demonstrated also that daily consumption of one-gram capsules of ginger powder for 8 weeks is useful for patients with type 2 diabetes as it reduced fasting blood glucose and glycated hemoglobin (HbA1c). This work is also in agreement with that of Khan *et al*. (2006), in which capsule of cloves were found to improve the function of insulin and to lower blood glucose and concluded that consumption of 1-3g of clove improve glucose level in diabetic patients. However, this work does not agree with the report of Ashade, *et al*. (2014), where ginger peel does not lower blood glucose levels in cat fish. The difference in the results may be as a result of method of preparation of ginger. The pronounced hypoglycaemic activity of the clove supplement may probably be also attributed to the activities of dehydrodieugenol and dehydrodieugenol B that had potent PPAR-γ ligand-binding activity, therefore causing increased insulin sensitivity via muffling the effect of lipotoxicity along insulin signaling pathway (Kuroda *et al*. 2012).

A significant increase in serum insulin level in high fat diet induced diabetic rabbits when compared to the normal rabbits was observed. Insulin activity may be low in these animals (diabetic control) due to the presence of excess FFAs in circulation that inhibit insulin signaling pathways. Consequent to this, higher plasma nutrients will be present in circulation that further leads to secretion of counter regulatory hormones such as glucagon and GLP1, which stimulates further secretion and release of insulin in to the circulation, resulting in hyperinsulinemia over time (Han *et al*., 2009). Treatment with fermented ginger also revealed a significant increase in insulin levels when compared to the level in diabetic control, fermented ginger may have exerted insulin mimetic effects thereby sparing serum insulin or may have contributed to increase insulin secretion and release. In the present study, the result of serum the insulin receptor level showed an increase similar to what was observe with serum insulin. The high fat diet could have induced insulin resistance as an underline mechanism leading to metabolic dysregulation in the animal model, resulting in the up-regulation of the insulin receptor, being a physiological response to sustained hyperglycaemia seen in pre-diabetic state or in diabetes with insulin resistance (Hyun *et al*., 2014). These findings could be explained by the proposed mechanism underlining the hypoglycaemic effect of ginger through increasing uptake of glucose into muscle cells without using insulin via regulating insulin inhibitory and stimulatory receptor proteins gene expressions (Arablou *et al*., 2014).

Serum Leptin level was significantly decreased in clove supplemented diet treated group compared with hyperglycaemic rabbits fed on normal feed in this study. Leptin, produced by adipocytes regulates energy expenditure, feeding behaviour and body weight. Abnormal higher levels of leptin are associated with hyperinsulinemia and insulin resistance (reference?). Leptin has a direct effect on glucose level independent of body weight and food intake (Kamohara *et al*., 1997). Nevertheless, leptin level is an indicator of better energy balance and control. It exerts an important role in regulation of glucose homeostasis; the mechanism of this effect may be at different level including glucose absorption, and as well secretion of insulin and down regulate counter regulatory hormones such as glucagon (Gabriela *et al*., 2015). So, the clove supplemented group may have an enhanced sensitivity effect on circulatory leptin which complements the down regulatory effect of the supplement on blood glucose level. Hence, the hypoglycaemic activity of the supplement may also be attributable to the physiological adaptive Leptin restoration effect on glucose homeostasis. Clove supplements could be acting centrally via Leptin effects on energy intake to down regulate blood glucose while the ginger supplements activity is likely at post receptor level via increased glucose transporters. In conclusion Clove, fermented ginger and their combined supplements treatments for six weeks improved glucose homeostasis and makers of energy balance in high fat diet induced type 2 diabetic rabbits.

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