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Research Article

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Effect of Ginger (*Zingiber officinale*) Roots Extract and its 6-Shogaol Compound in Obese Diabetic Rat Model

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Abstract *Background:* Ginger (*Zingiber officinale*, family *Zingiberaceae*) is a perinneal plant whose its roots are used as a spice and for medicinal purposes. *Aim:* To investigate some pharmacological effects of ginger roots extract (GE) and its 6-Shogaol (6-SHO) compound in obese diabetic rat model. *Materials and Methods:* Sixty mature rats were allocated into 6 groups (n=10). Group 1 was kept negative control and the other 5 groups were fed high-fat diet (HFD) for 4 weeks to induce obesity. The obese rats were rendered diabetic by daily subcutaneous injection of 120 mg/kg alloxan for 5 days. After induction of diabetes, group 2 was kept positive control (obese diabetic) and the remaining 4 groups were orally given GE in 100 and 200 mg/kg and 6-SHO in 5 and 10 mg/kg daily for 6 weeks, respectively. Blood samples were collected for biochemical analysis and livers were dissected out for preparing homogenates. *Results:* GE and its 6-SHO decreased body and fats weights and levels of aspartate aminotransferase (AST), alanine aminotransferase enzymes (ALT), total cholesterol (TC), triglycerides (TG) and low-density lipoprotein (LDL). Blood glucose and leptin were decreased, while insulin was increased in rats given GE and its 6-CHO. The activity of hepatic superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes was increased. Conclusion: GE produces an anti-obesity, hepatoprotective, hypolipidemic, antidiabetic, and anti-oxidant effects. These effects may be attributed to the presence of 6-SHO compound in GE. Ginger, a popular herb used as a drink, may be beneficial for obese diabetic patients.

Keywords Ginger, anti-obesity, hepatoprotective, hypolipidemic, antidiabetic, anti-oxidant

Introduction

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia due to insulin deficiency, or insulin resistance, or both. Hyperglycemia occurs when the cells become unable to utilize glucose and/or the liver and skeletal muscles cannot store glycogen [1]. The high blood glucose concentrations result in oxidative stress due to increased production of reactive oxygen species (ROS) and sharp decrease in antioxidant body defense mechanisms [2]. Oxidative stress plays a key role in the onset and development of diabetes complications, notably diabetic nephropathy [3]. Because the synthetic chemical drugs used for treating obesity and diabetes have many adverse side effects. Therefore, there is a great need to search for alternative safe natural agents from medicinal plants and herbs.

Obesity is an excessive fat accumulation in the body which results from an imbalance between energy intake and energy expenditure. It is associated with genetic, metabolic, and behavioral components. Despite of a major contribution of genetic susceptibility, the rapid development of obesity might reflect great changes of the other

factors such as a dietary habit [4]. The prevalence of obesity is increased dramatically among all ages due to changes of lifestyles and dietary fat intake [5]. Obesity represents a serious health problem that increased the risk for many diseases such as hypertension and diabetes mellitus [6]. Obesity and diabetes are among the most challenging global health problems. There is a strong association between obesity, insulin resistance, and infiltration of the adipose tissues by inflammatory cells. Insulin resistance, that is a common accompaniment of obesity, is a major risk factor for diabetes mellitus [7].

Ginger (*Zingiber officinale*, family *Zingiberaceae*) is a flowering perinneal plant whose its roots are widely used as a popular culinary spice and in folk medicine, Ginger was first cultivated in Asia and has a long history in medicinal benefits for the treatment of pain, inflammation, osteoarthritis, stomach ache, nausea, and several types of human cancers [8], [9], [10] and [11]. Ginger roots contain several pungent constituents such as gingerols, shogaols, paradols and gingerdiols beside some phenolic ketone derivatives [12]. Ginger roots are used medicinally for its hepatoprotective and antioxidant [13], antidiabetic and hypolipidemic [14], [15] and anti-obesity [16] effects. Ginger is widely used in traditional medicine for its anti-inflammatory, analgesic, antipyretic and antimicrobial activities [17]. Recently, 6-Paradol, the major metabolite of 6-Shogaol, significantly reduced blood glucose, cholesterol and body weight in high-fat fed mice [18] and [19]. The ginger compound 6-Shogaol (6-SHO) has been studied for its antitumor, anti-inflammatory and neuroprotective effects [20].

The present study aimed to investigate some pharmacological effects of ginger roots extract and its 6-Shogaol compound in obese diabetic rats.

Materials and Methods

Rats

Sixty adult male Sprague-Dawley rats (240-250 g B.wt. and 9-10 weeks old) were used in this study. Animals were obtained from Laboratory Animal Colony, Agricultural Research Center, Giza, Egypt. Rats were housed under hygienic conditions at 24 °C temperature, 50% relative humidity and 12 hrs light/12 hrs dark cycles. Basal diet and water were freely provided.

Ginger Roots and its 6- Shogaol Compound

Dried barks of ginger roots were purchased from a local market of Agricultural Herbs, Spices and Medicinal plants, Cairo, Egypt. The plant was authenticated in Botany Department, Faculty of Science, Cairo University. The dried plant materials were grinded using an electric mixer into a fine powder and subjected to aqueous extraction.

Shogaols are found in only small quantities in the fresh roots and are mainly found in the dried and thermally treated roots 6-Shogaol is the most abundant bioactive constituent in ginger roots. 6-Shogaol compound was obtained as an analytical standard grade from Sigma-Aldrich Company, Cairo branch.

Alloxan and Biochemical Kits

Alloxan was purchased from El-Gomhoryia Company for Chemicals, Cairo, Egypt as white powder packed in bottles each of 25-gram alloxan monohydrate. Glucose enzymatic kits for estimating blood glucose and radioimmunoassay kits for estimating leptin and insulin were purchased from Gamma Trade Company, Egypt. The other kits were obtained from Biodiagnostics Company, Egypt.

Preparation of Basal Diet

The dietary supply of protein, fat, carbohydrates, vitamins and minerals was in accordance with the recommended dietary allowances for rats [21]. Basal diet is consisted of 20 % protein, 10 % sucrose, 5 % corn oil, 2% choline chloride, 1% vitamin mixture, 3.5 % salt mixture and 5% fibers (cellulose). The remainder was corn starch up to 100 %.

Preparation of Ginger Aqueous Extract

Five hundred (500) grams of the dried powder of ginger roots were added to two (2) liters distilled water and boiled for 10 minutes, cooled and filtered to obtain 25 % aqueous extract. A voucher sample number (GE # 111) was deposited in the Pharmacology Department, Faculty of Veterinary Medicine, Cairo University.

Induction of Obesity and Diabetes

Obesity was induced by feeding rats on high-fat diet (HFD) which supplies 45 % calories from fat (pig lard) for 4 weeks. A four-week HFD feeding is sufficient to induce obesity and acute hyperlipidemia. This obese model of rats is closely resembling the reality of obesity in man [22]. The obese rats were then rendered diabetic S/C of alloxan in a dose of 120 mg/kg daily for 5 days [23].

Experimental Design

Sixty mature male Sprague Dawley rats were randomly distributed into 6 groups, of 10 rats each. Group 1 was fed on basal diet and kept as negative control, while the other 5 groups were fed on HFD for 4 weeks to induce obesity. The obese rats were then rendered diabetic by S/C of alloxan (120 mg/kg/day) for 5 days. Group 2 was kept obese diabetic (positive control), while the other groups 3, 4, 5 and 6 were orally given GE in doses of 100 and 200 mg/kg and 6-SHO in doses of 5 and 10 mg/kg daily for 6 weeks, respectively. At the end of feeding period, the initial and final body weights of rats were recorded and body fats were carefully removed and weighed. The adiposity index (Ad. I) was calculated as Ad. I = fats weight/body weight x100. Rats were then anesthetized by intraperioneal dose 50 mg/kg of pentobarbital sodium and blood samples were withdrawn by puncture of orbital plexuses veins of eye. Blood was left to clot and centrifuged for 10 minutes for separating the serum which kept frozen until biochemical analyses. Livers were dissected out for estimating the activity of tissue antioxidant enzymes in liver homogenates. All procedures were carried out according to the guidelines of the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Cairo University, Egypt.

Biochemical Analysis

Serum AST and ALT [24], total cholesterol [25], triglycerides (TG) [26] and high-density lipoprotein cholesterol [26] were chemically determined using specific biochemical kits and measured using a spectrophotometer. Low density lipoprotein (LDL-c) cholesterol was calculated using Friedewald formula (FF): LDL-c = (TC) - (HDL-c) - (TG/5). Blood glucose (BG) was determined using glucose enzymatic kits [27]. Insulin was estimated using specific antibody radioimmunoassay (RIA) [28]. Leptin hormone was determined using enzyme-linked immunosorbent assay [29].

Assessment of Antioxidant Enzymes

One gram of liver tissue was washed with ice-cooled 0.9% NaCl solution and homogenized in 100 ml of ice-cooled 1.5% solution of potassium chloride and 50 mmol potassium phosphate buffer solutions (pH 7.4) to yield 1% homogenate (w/v). Homogenization was performed using Sonicator, 4710 Ultrasonic Homogenizer. Liver homogenates were centrifuged at 4000×g for 10 min. at 4°C and the supernatants were used to assay the activity of antioxidant enzymes glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) according to [30], [31] and [32], respectively.

Statistical Analysis

Data were presented as means ± SD and analyzed with a one-way analysis of variance (ANOVA) test using GraphPad (Prism).

Results

Effect on Body and Fat Weights

Feeding of rats on high-fat diet (HFD) for 4 weeks significantly ($P < 0.05$) increased the body weight (B.wt.), fats weight and adiposity index (Ad. I) as compared to the negative control group. Oral administration of GE (100 and 200 mg/kg) and its 6- SHO (5 and 10 mg/kg) to obese diabetic rats for 6 weeks caused significant ($P < 0.05$) decreases in B.wt., fats weight and Ad. I as compared to the obese diabetic rats as depicted in Table 1.

Effect on Serum Liver Enzymes

Rats fed on HFD for 4 weeks had significant (P < 0.05) increases in serum levels of AST and ALT as compared to the negative control group. Administration of GE (100 and 200 mg/kg) and its 6-SHO (5 and 10 mg/kg) to obese diabetic rats significantly (P< 0.05) reduced the high serum levels of AST and ALT as compared to the positive control group (**Figure 1**).

Table 1: Effects of ginger aqueous extract (GE) and its 6 shogaol (6-SHO) compound on body weight (B.wt.), fats weight and adiposity index (Ad. I) in obese diabetic rats. (n= 10)

Parameters Groups	B.Wt. (g)	Fat Wt. (g)	Ad. I (%)
Negative control	245.0±11.0^{d}	6.55±0.12^{c}	2.67±0.10^{d}
Positive control	300.0±12.5^{a}	14.50±0.52^{a}	4.83±0.35^{a}
GE (100 mg/kg)	288.0±10.5^{b}	10.20±0.45^{b}	3.54±0.24^{b}
GE (200 mg/kg)	278.0±11.0^{c}	9.50±0.37^{c}	3.41±0.22^{b}
6-SHO (5 mg/kg)	280.0±12.5^{b}	8.40±0.30^{b}	3.00±0.25^{c}
6-SHO (10 mg/kg)	270.0±11.0^{c}	7.80±0.15^{c}	2.88±0.12^{c}

Mean ± SD with different letters superscripts (a, b, c, d) in the same column is significant at P < 0.05 using one-way ANOVA test

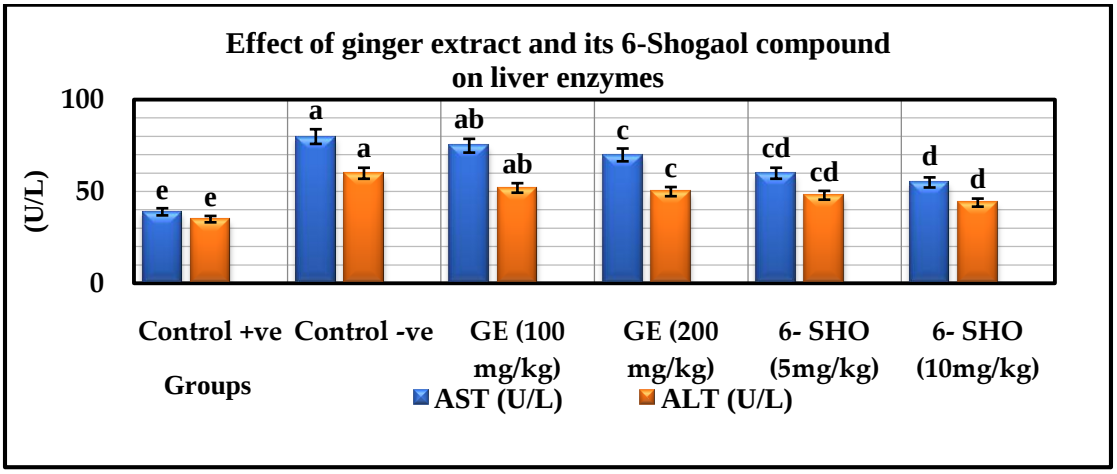

Figure 1: Effect of ginger aqueous extract (GE) and its 6-shogaol (6-SHO) compound on serum aspartate aminotransferase (AST) and alanine transferase (ALT) in obese diabetic rats

Effect on Serum Total Cholesterol and Triglycerides

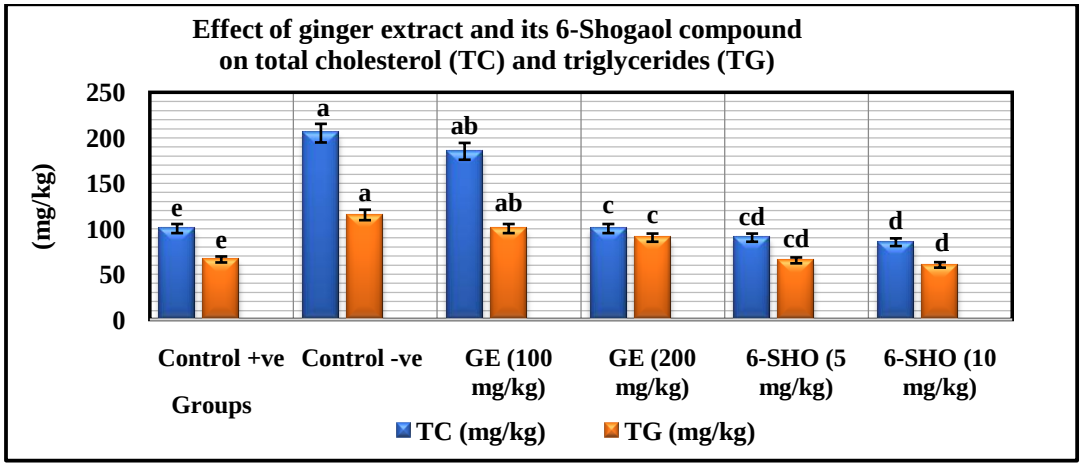

Figure 2: Effects of ginger aqueous extract (GE) and its 6-shogaol (6-SHO) compound on serum total cholesterol (TC) and triglyceride (TG) in obese diabetic rats

Feeding of rats on HFD for 4 weeks significantly (P<0.05) increased serum total cholesterol (TC) and triglycerides (TG) as compared to rats fed on basal diet. Administration of GE (100 and 200 mg/kg) and its 6-SHO (5 and 10 mg/kg) to obese diabetic rats for 6 weeks significantly (P<0.05) decreased the elevated serum TC and TG levels as compared to the positive control group (Figure 2).

Effects on Serum High- and Low-Density Lipoproteins

Feeding of rats on HFD for 4 weeks significantly (P <0.05) decreased serum high-density lipoprotein (HDL-c), increased low density lipoprotein (LDL-c) and atherogenic index (AI) as compared to the negative control rats. Administration of GE (100 and 200 mg/kg) and its 6-SHO (5 and 10 mg/kg) to obese diabetic rats for 6 weeks significantly (P <0.05) increased serum HDL-c but decreased LDL-c and AI as compared to the positive control group (Table 2).

Table 2: Effects of ginger aqueous extract (GE) and its 6-Shogaol (6-SHO) compound on serum levels of high-density lipoprotein (HDL-c), low density lipoprotein (LDL-c) cholesterol and atherogenic index (AI) in obese diabetic rats. (n=10)

Parameters Groups	HDL-c (mg/kg)	HDL-c (mg/kg)	AI (%)
Negative control	69.40±3.11^{a}	12.50±2.41^{d}	0.180
Positive control	54.34±2.55^{c}	60.50±4.15^{a}	0.738
GE (100 mg/kg)	60.66±3.22^{b}	44.80±3.25^{b}	0.738
GE (200 mg/kg)	62.45±4.12^{b}	33.20±2.27^{c}	0.531
6-SHO (5 mg/kg)	66.50±3.16^{c}	31.45±3.19^{c}	0.472
6-SHO (10 mg/kg)	68.50±4.16^{c}	25.22±3.16^{c}	0.368

Mean ± SD with different letters superscripts (a, b, c, d) in the same column is significant at P < 0.05 using one-way ANOVA test.

Effect on Blood Glucose, Leptin and Insulin concentrations

Data reported in **Table 3** showed that rats fed on HFD for 4 weeks had significant (P < 0.05) increases in blood glucose and leptin hormone and a decrease in insulin hormone levels as compared to rats fed on basal diet. GE (100 and 200 mg/kg) and its 6-SHO (5 and 10 mg/kg) when orally given to obese diabetic rats for 6 weeks significantly (P <0.05) decreased blood glucose and leptin hormone, but increased insulin levels as compared to the positive control group.

Table 3: Effects of ginger aqueous extract (GE) and its 6 shogaol (6-SHO) compound on serum levels of blood glucose (BG) and leptin and insulin concentrations in obese diabetic rats. (n=10).

Parameters Groups	BG (mg/dL)	Leptin (ng/ml)	Insulin (ng/ml)
Negative control	190±12.0^{c}	2.50±0.15^{d}	2.95±0.15^{a}
Positive control	285±10.0^{a}	4.90±0.11^{a}	0.89±0.13^{d}
GE (100 mg/kg)	266±13.0^{b}	4.10±0.18^{b}	1.82±0.24^{b}
GE (200 mg/kg)	245±11.0^{b}	3.35±0.17^{b}	2.43±0.12^{b}
6-SHO (5 mg/kg)	227±10.0^{c}	2.75±0.19^{c}	2.55±0.14^{c}
6-SHO (10 mg/kg)	215±10.0^{c}	2.80±0.12^{c}	2.65±0.11^{c}

Mean ± SD with different letters superscripts (a, b, c, d) in the same column is significant at P < 0.05 using one-way ANOVA test.

Effect on liver Antioxidant Enzymes

Feeding HFD to rats for 4 weeks significantly (P < 0.05) decreased hepatic tissue levels of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes as compared to the negative control group. Oral administration of GE (100 and 200 mg/kg) and its 6-SHO (5 and 10 mg/kg) to obese diabetic rats

normalized the elevated hepatic tissue levels of SOD, GPx and CAT as compared to the positive control group (Table 4).

Table 4: Effects of ginger aqueous extract (GE) and its 6-shogaol (6-SHO) compound on activities of liver superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes in obese diabetic rats. (n=10).

Parameters Groups	SOD (U/mg protein)	GPx (nmol/min/ mg protein)	CAT nmol/min /mg protein)
Negative control	58.70±2.24[a]	0.69±0.01[a]	0.185±0.001[a]
Positive control	38.50±2.88[d]	0.18±0.04[d]	0.138±0.002[d]
GE (100 mg/kg)	44.74±3.46[c]	0.32±0.03[b]	0.145±0.001[b]
GE (200 mg/kg)	48.95±2.58[c]	0.34±0.01[b]	0.158±0.001[b]
6-SHO (5 mg/kg)	56.25±2.73[b]	0.49±0.01[c]	0.175±0.001[c]
6-SHO (10 mg/kg)	59.25±2.33[b]	0.52±0.02[c]	0.180±0.002[c]

Means ± SD with different letters superscripts (a, b, c, d) in the same column is significant at $P < 0.05$ using one-way ANOVA test.

Discussion

The medicinal plants and culinary herbs which possess antihyperlipidemic and antidiabetic activities have gained much attention, especially those with little toxic properties. The biological value of plants depends on their bioactive constituents such as saponins, anthocyanins, flavonoids, polyphenols, diterpenes, triterpenes and other phytochemicals [33].

Obesity, especially the abdominal type, is a health problem that causes a metabolic disease and increases the incidence of diabetes mellitus, hypertension, dyslipidemia, and atherosclerosis. Oxidative stress, which increased in obesity, plays an important role in the development of diabetes and cardiovascular diseases in people who are obese. The increased oxidative stress together with the decreased antioxidative defense seems to contribute to decreased insulin sensitivity and impaired insulin secretory response in obese diabetics, and favours the development of diabetes during obesity [34].

In this study, obesity was induced by feeding rats on high-fat diet (HFD) for 4 weeks according to the method described [22]. This model of obesity in rats closely resembles the reality of obesity in humans. However, experimental obesity could be also induced in rats and mice by other methods such as feeding on high-fructose diet, damage in the anterior hypothalamus and genetically induced-obesity. In the present study, the used rat model was obese diabetic where the obese rats were rendered diabetic by intraperitoneal injection of alloxan for 5 days [23]. GE and its 6-SHO produced an anti-obesity effect in obese diabetic rats. This effect was in accordance with that reported by [35]. This author concluded that ginger has a great ability to reduce body weight in rats fed high-fat diet.

The mechanism(s) underlying the anti-obesity effect of GE and its 6-SHO could be possibly explained by its hyperinsulinemia that was reported in the present study. The hyperinsulinemia and insulin resistance are common features of obesity in humans and experimental animals [36]. The anti-obesity effect of GE and its 6-SHO could also be due to the high level of leptin hormone reported the current study. It is known that leptin is a peptide hormone secreted by adipose tissue in proportion to its mass. Leptin circulates in the blood and acts on the brain to regulate food intake (appetite) and energy expenditure. When body fat mass decreases, the plasma leptin levels decrease so stimulating appetite and suppressing energy expenditure till fat mass is restored. On this basis, the reduced adiposity index in rats given GE and its 6-SHO could be attributed to the reported low serum leptin level.

The hepatoprotective effect of GE and its 6-SHO reported in this study was evident from the significant decreases in the elevated serum levels of liver enzymes AST and ALT in obese diabetic rats. This effect agreed with the previously reported [37], [38]. The hepatoprotective effect of GE and its 6-SHO could be attributed to the antioxidant activity of ginger [37].

The decreases in serum levels of TC, TG and LDL-c caused by GE and its 6-SHO, in this study, were similar to those previously recorded [14]. The previous authors concluded that GE lowers the elevated levels of TC, TG and LDLc in man and rats. The hypolipidemic effect of GE could be attributed to its contents of gingerols and shogaols which inhibit the intestinal absorption of cholesterol or inhibit cholesterol synthesis in the liver so reduce serum cholesterol levels.

The hypoinsulinemic effect of GE and its 6-SHO in obese diabetic rats reported in the current study was similar to the recently reported [20]. Previous studies revealed that hyperinsulinemia and insulin resistance were common features of obesity in rats [36].

The high leptin level in rats was similar to that previously reported [39] who found that HFD increased serum leptin level in rats. Leptin plays a key role in regulating energy intake and energy expenditure and the level of circulating leptin is proportional to the total amount of body fats. GE and its 6-SHO significantly decreased serum leptin levels in obese diabetic rats. This finding agreed with the previously reported that ginger decreased 6% to 16% in leptin levels in humans

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