Ginger Enhances Antioxidant Activity and Attenuates Atherogenesis in Diabetic Cholesterol-Fed Rats

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Abstract: Ginger effects on blood glucose, lipids, oxidative stress and early stages of atherogenesis in diabetic cholesterol-fed rats were investigated in the present study. Sprague-Dawley male rats were divided into three main groups of eight animals each. The animals of the first group were fed on a commercial pellet diet and served as control. Diabetes was induced in both second and third group of animals by intraperitoneal injection with one dose of streptozotocin (60 mg/kg) and fed on the same pellet diet mixed with 0.5% cholesterol. The animals of the third group were daily administered by ginger (25 mg/kg body weight) using oral gavages. After six weeks of the experimental period, different blood lipid parameters, lipid peroxidation and anti-oxidant activities were analyzed in all groups. Samples of aorta were obtained to investigate the vascular wall changes using electron microscopy (EM) studies. The results revealed that ginger produced a decline in blood glucose and significant decrease of triglyceride levels, but could not restore the increase of cholesterol and low density lipoprotein cholesterol levels of diabetic cholesterol-fed rats. Ginger administration resulted in significant reduction in malondialdehyde concentration and significant elevation in total plasma antioxidant activity. Furthermore, EM studies revealed that ginger ameliorated the cellular changes of aortic wall induced in diabetic cholesterol-fed animals. The data of the present study indicated that antitherogenic effect of ginger could be attributed to its vital role in regulation of per-oxidation process, antioxidant activity and inhibition of monocyte migration and interaction accompanied with endothelial dysfunction.

Key words: Diabetes; Vascular disorders; Lipid peroxidation; Ginger; Antioxidants.

INTRODUCTION

Diabetes is associated with a number of metabolic alterations, of which hyperglycemia and lipid disorders are principal. Hyperglycemia induces the overproduction of oxygen free radicals and consequently increases lipid and protein oxidation (Ramarkrishna and Jailkhah, 2007). Dyslipidemia was suggested as a primary risk factor for development of atherosclerosis in diabetic patients (Laakso, 1995). Although atherosclerosis is considered a multi-factorial disease which may be induced by many mechanisms, the deposition of lipids in the arterial wall, endothelial cell injury, platelet adherence and release of growth factors and subsequent entry of monocytes are initial events observed during atherogenesis (Ross, 1981; Nilsson, 1986; Masuda and Ross, 1990). Either endogenous or exogenous hypercholesterolemia was found to initiate the generation of free radicals and points out their role in damage of essential macromolecules (Pappolla et al., 2002). Moreover, It was reported that oxidative stress and ROS (reactive oxygen species) increase in patients with diabetes mellitus and suggested to induce endothelial cell dysfunction and trigger the progression of atherosclerosis (Nakagami et al., 2005).

Oxidative stress has been reported to be a major factor in the pathogenesis of all diabetic complications. Free radicals are formed in diabetes by glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes and increased lipid peroxidation (Dominic et al., 2002). It was also indicated that low density lipoprotein cholesterol and its oxidative modification are major determinants of endothelial dysfunction and oxidative stress in patients with coronary artery disease (Al-Benna, 2006). However, increasing evidence suggests that lowering oxidative stress and activation of antioxidant defense mechanisms, using medicinal plants and dietary antioxidants, prevents endothelial dysfunction and cell damage in diabetes, atherosclerosis and cardiovascular diseases.

Ginger (Zingiber officinale) has been used since the ancient times as a spice and medicine in Asian and Arabic herbal traditions. It has been used in a wide variety of diseases, especially in gastrointestinal disorders, such as constipation, diarrhea, anorexia, colic, dyspepsia, nausea, vomiting, and motion sickness (Langner et al., 1998). The hypotensive, vasodilator, anti-inflammatory, antitumor, antilipidemic and antioxidant pharmacologic effects of ginger have been reported (Thomson et al., 2002; Chrubasik et al., 2004). On the other hand, Bordia et al. (1997) reported that ginger did not affect blood sugar and lipids. Similarly, Weidner and Sigwart (2000)
found no significant effect of ginger on blood glucose, blood pressure, heart rate and coagulation parameters. More recent investigations stated that ginger induced hypoglycemic and hypolipidemic effects in streptozotocin induced diabetic rats (AL-Amin et al., 2006). Other studies suggested that the response to ginger components depends on its dose concentration (Ghayur et al., 2005; Siddaraju and Dharmesh, 2007). The medicinal properties of ginger were attributed to many active compounds in ginger. The major constituents in Zingiber officinale are the pungent vanilloids, -gingerol, -paradol, shogaols and zingerone (Lee amd Surh, 1998; Zick et al., 2008). The antioxidant, antitumor, and anti-inflammatory pharmacologic effects of ginger were mainly attributed to these constituents (Zick et al., 2008).

Some experimental data published on antidiabetic, hypolipidemic and antioxidative properties of ginger are controversial and more investigations may clarify its potency in protection and treatment of metabolic disorders. Therefore, the present study was undertaken to evaluate the effect of ginger administration on some biochemical parameters and vascular wall alterations induced by experimental diabetes and lipid disorders in rats.

MATERIALS AND METHODS

Experimental Design:
Adult male albino rats of Sprague–Dawly strain (120 – 150 g body weight) were used in the present study. The animals were divided into three main experimental groups, of eight rats each. Animals of the first group (G1) were fed a normal commercial pellet diet and served as control. Diabetes was induced in animals of the second (G2) and third (G3) groups by intraperitoneal injection of streptozotocin (60 mg/kg). Rats of G2 and G3 groups received a diet of normal pellet mixed with (0.5%) cholesterol. This diet was prepared by spraying normal pellets with cholesterol dissolved in ethyl-ether and allowing the solvent to evaporate. Only rats of group (3) were daily administered by ginger (25 mg/kg body weight) using oral gavages.

All animal groups were maintained for a treatment period of six weeks. At the end of experimental period, the animals were fasted overnight and then sacrificed to obtain blood and aortic samples. Samples of heparinized blood were separated and stored at 4°C, while remaining blood was used for preparation of plasma and serum and immediately stored at -85°C until use for biochemical analysis. Samples of thoracic aorta were cut open to expose the luminate surface, fixed in cold glutaraldehyde (5%) for 24 hours and prepared for investigation by scanning (SEM) and transmission electron microscopy (TEM).

Biochemical Analysis:
The levels of serum glucose, triglycerides total cholesterol, LDL-cholesterol and HDL-cholesterol were analyzed using Biodiagnostic kits according to the methods of Trinder (1969), Fassati, P. and Prencipe, L. (1982), Allain et al. (1974), Wieland and Seidel (1983) and Lopez-Virella et al., (1977) respectively. Plasma lipid peroxidation (malondialdehyde concentration) and total antioxidant capacity were determined according to Satoh (1978) and Koracevic et al. (2001). Reduced blood glutathione concentration was determined according to the method of Beutler et al. (1963).

Preparation Of Samples For Electron Microscopy:
The glutaraldehyde-fixed aortic specimens were washed in cacodylate buffer (pH 7.2), post-fixed in cold 1% osmic acid for 2 hours and then washed in cacodylate buffer. Dehydration was done by using ascending grades of ethanol. Each specimen was devided into two parts: The first was dried from CO2, coated with gold and examined by SEM. The second was embedded in Epon 812 using gelatin capsules, sectioned with ultramicrotome contrasted in uranyl acetate and lead citrate, and examined by transmission electron microscope.

Statistical Analysis:
The data were statistically analyzed by one-way analysis of variance (Anova- Tukey test) using statistical package for the social sciences (SPSS) program (SPSS Inc., version 10). All data were expressed as mean ± standard deviation (S.D.). The probability value less than 0.05 was taken as the level of statistical significance.

Results:
As is apparent from the data presented in table (1) and figure (1-A), a significant increase (P< 0.05) in plasma glucose concentration in treated groups as compared to control group (G1).On the other hand, a decrease of blood glucose in ginger-administered animals (G3) was found as compared to diabetic cholesterol-fed rats (G2), but this change was not statistically significant. Moreover, a significant increase in plasma triglycerides (Fig. 1-B), total cholesterol (Fig. 1-C), LDL-cholesterol (Fig. 1-D) and HDL-cholesterol (Fig. 1-E) was observed in diabetic cholesterol-fed animals as compared to control group (G1). However, ginger induced a significant decrease in plasma triglycerides level (G3) as compared to that of G2, but this lowering effect was not significant in relation to cholesterol parameters.
Elevated levels of plasma malondialdehyde concentration, as an indicator of lipid per-oxidation process, were found in diabetic cholesterol-fed group. Administration of ginger lowered significantly the level of lipid peroxidation in diabetic cholesterol-fed rats, but still higher than normal control group (Fig. 1-F). A marked significant decrease in blood reduced glutathione concentration was observed in treated groups, but this decrease was reduced in ginger treated animals (Fig. 1-G). Plasma total antioxidant capacity decreased significantly in G2 as compared to G1, but ginger administration to G3 rats ameliorated its level and showed a significant increase as compared to that of G2 (Fig. 1-H). However, it is apparent from the data presented that the lowering effects of ginger on lipid peroxidation and its antioxidative property are more potent than its modulating action on glucose and other lipid parameters.

Electron microscopic studies revealed changes of endothelial cells, monocytic adhesion and penetration to endothelial cells and alterations in subendothelial space in aorta of diabetic cholesterol-fed rats. Scanning electron micrographs demonstrated alterations in the shape and direction of endothelial cells when compared to the elongated, elliptical and flattened appearance of endothelial cells of control animals. Swellings of endothelial cells with adhesion of monocytes were observed. Also, surface of endothelial cells appeared covered by microvilli and long projections and other cells were perforated with numerous craters. Some disrupted and desquamated endothelial cells could also be observed in aortas of G2 rats (Fig. 2A-F).

Transmission electron micrographs showed also changes in endothelium and subendothelial space of aortas of G2 animals as compared to control. Vacuolation of the endothelial cells and appearance of fragile cytoplasmic processes often projected from the lumen surface and disrupted cells were frequently found in the endothelium overlying accumulations of foamy macrophages. A perceptible thickening of subendothelial space could be observed in aortas of normal animals (G1). Ginger administration induced less number of adhered monocytes to the inner layer of vascular wall. Moreover, SEM showed that endothelial craters and ruptured endothelial cells decreased markedly as compared to group G2 (Fig. 2G&H).

The results revealed by transmission electron micrographs showed that ginger supplementation to diabetic cholesterol-fed rats induced less widespread of vacuolation in endothelial cells, decrease of adhered monocytes and less fragmentation of elastic lamina as compared to G2. Moreover, the presence of macrophages in the intimal layer diminished in ginger treated animals (Fig. 3E&F). It seems therefore that electron microscopic studies revealed minimized formation of early atherogenic changes, amelioration of deleterious cellular alterations and delaying vasculopathy in diabetics under the effect of ginger treatment during the early stages of the disease.

**Discussion:**

In the present study, diabetic cholesterol-fed animals showed some metabolic disorders similar to those previously reported in studies on experimental animals and diabetic patients. Hyperglycemia, dyslipidemia, oxidative stress, endothelial cell turnover and changes of vascular wall were observed in this study. Hyperglycemia, the most striking change characterizing metabolic disorders in diabetics, has been shown to be strongly associated with lipid disorders and results in glycosylation of proteins and production of more reactive oxygen species; the conditions which may damage many cellular biomolecules such as lipids, proteins, carbohydrates and nucleic acids (Hannon-Fletcher et al., 2000; Siti-Balkis et al., 2008).

Abnormalities of blood lipid profile represented by the rise of triglyceride and cholesterol levels in plasma of diabetic cholesterol-fed rats were also observed in the present study. This rise was attributable to both endogenous changes of lipid metabolism caused by diabetes and feeding animals on cholesterol-rich diet.

### Table 1: Mean levels (+ Standard deviation) of different blood biochemical parameters in control and treated rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group (g1)</th>
<th>Diabetic Cholesterol-fed Group (g2)</th>
<th>Ginger-treated Diabetic Cholesterol-fed Group (g3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose concentration (mg/dl)</td>
<td>99.06 ± 6.84</td>
<td>225.78 ± 27.10</td>
<td>219.207 ± 16.79</td>
</tr>
<tr>
<td>Serum triglyceride concentration (mg/dl)</td>
<td>64.05 ± 5.11</td>
<td>117.73 ± 20.52</td>
<td>92.35 ± 14.72</td>
</tr>
<tr>
<td>Serum total cholesterol concentration (mg/dl)</td>
<td>74.75 ± 8.89</td>
<td>132.05 ± 22.45</td>
<td>122.20 ± 17.47</td>
</tr>
<tr>
<td>Serum LDL-cholesterol concentration (mg/dl)</td>
<td>31.01 ± 4.28</td>
<td>63.13 ± 19.38</td>
<td>60.62 ± 10.96</td>
</tr>
<tr>
<td>Serum HDL-cholesterol concentration (mg/dl)</td>
<td>35.24 ± 7.52</td>
<td>53.40 ± 13.47</td>
<td>51.55 ± 10.79</td>
</tr>
<tr>
<td>Plasma lipid peroxidation (Malondialdehyde concentration (nmol/ml)</td>
<td>2.15 ± 0.93</td>
<td>4.72 ± 2.08</td>
<td>2.86 ± 0.96</td>
</tr>
<tr>
<td>Blood reduced glutathione concentration (mg/dl)</td>
<td>16.23±2.97</td>
<td>9.14±2.23</td>
<td>11.86±2.33</td>
</tr>
<tr>
<td>Plasma total antioxidant capacity (mM/L)</td>
<td>1.04±0.242</td>
<td>0.43±0.163</td>
<td>0.801±0.324</td>
</tr>
</tbody>
</table>

* Significant difference as compared to G1 (p<0.05). # Significant difference as compared to G2 (p<0.05).
Similar observations were reported in previous studies (Stout, 1979; Garcia-Frade et al., 1987; Taskinen et al., 1988; Nodestgard and Zilversmit, 1989) which attributed the blood lipid changes in diabetics to increased production of very low density lipoprotein (VLDL) and reduced receptor-mediated uptake and degradation of low density lipoprotein (LDL) by liver cells. It is generally accepted that low density lipoprotein (LDL) is the main atherogenic lipoprotein and LDL-cholesterol is therefore the primary target for lipid lowering therapy (Schaefer et al., 1995).

![Bar graphs of mean levels of [A] serum glucose, [B] triglycerides, [C] total cholesterol, [D] LDL-cholesterol, [E] HDL-cholesterol, [F] Plasma lipid peroxidation, [G] Blood reduced glutathione concentration and [H] plasma total antioxidant capacity. G1 is the control group. G2 is the group of diabetic cholesterol-fed rats. G3 is the group of ginger-treated diabetic cholesterol-fed rats. * indicates significant difference versus G1 while # indicates significant difference versus G2 (p<0.05).]

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Although the role of triglycerides as an independent risk factor for vascular disease has been a matter of debate, accumulating evidence indicates that elevated fasting TG levels are an independent risk factor for vascular disease (Hokanson and Austin, 1996; Assmann et al., 1998; Ridker et al., 2008). Therefore, triglycerides may work in concert with the other lipid variables to increase vascular risk. The increase of triglyceride levels in diabetic animals in the present study and earlier studies on diabetic subjects may therefore considered a risk factor for atherosclerosis in diabetics. This may agree with the finding that the risk of myocardial infarction was greatest among subjects with the highest tertile for both triglycerides and total cholesterol (Evagelos et al., 2005). Ryu et al., (1992) reported that prolonged exposure of arterial wall cells to triglyceride-rich chylomicron remnants enhances the atherogenic process. Moreover, Ridker et al. (2008) considered that measurement of non-fasting triglycerides rather than fasting triglycerides are indicators for atherogenecity and cardiovascular risk.

**Fig. 2:** Scanning electron micrographs showing [A] squamous and flattened appearance of aortic endothelial cells of control group (X 750). [B] Swelling of endothelial cells, adhesion of monocytes (thin arrow) and appearance of some craters (thick arrow) in endothelial cells of aortas of G2 group. [C] Detaching of some endothelial cells (thin arrow) and disruption of others (thick arrow) (X 750). [D] Large magnification showing some extended long projections (thin arrow) and craters (thick arrow) of endothelial cells in G2 group (X 3500). [E] Aggregation of monocytes on some areas of the inner surface of aortic wall (thick arrow). [F] Endothelial desquamation with adhered blood elements (thick arrow). [G&H] Inner layer of vascular wall of G3 group displaying less ruptured endothelial cells (thick arrow), and appearance of squamous uniform endothelial cells (thin arrows) with less craters and less number of adhered monocytes (X750).
However, the increase of different blood lipids has been found to be associated with an increase in endothelial cell turnover and early lesions of arterial wall, characterized by increased endothelial permeability, injury, adherence and penetration of blood–borne monocytes (Nilsson, 1986; Ceriello and Motz, 2004; Albenna et al., 2006). These alterations of arterial wall were also observed in diabetic cholesterol-fed animals in the present study, as recognized by electron microscopy.

![Fig. 3: Transmission electron micrographs showing: [A] Normal endothelial cell (E), elastic lamina (EL), and smooth muscle cell (S) in aortic wall of control animals. [B] Adhered monocyte (M) to endothelium, intimal monocyte (IM) and thickening of subendothelial space and EL in aortic wall of G2 group. [C] Vacuolated and injured endothelial cells (IE) and formation of irregular fragmented and granular internal elastic lamina (EL) in G2 group. [D] Blood borne monocyte (BM), injured endothelium (IE) and intimal foamy macrophages (Ma) with variant degree of vacuolation in G2 group. [E&F] less fragmentation of elastic lamina with regular appearance and decrease of adhered monocytes and intimal macrophages in aortic wall of rats of G3 group (X 4000).](image)

Most studies linking dyslipidemia to initiation and progression of atherosclerosis attributed this effect to oxidized low density lipoproteins which has been found to be cytotoxic to endothelial cells and chemotactic for monocytes. The increase of lipid peroxidation and decrease of total antioxidant capacity in diabetic rats of this study indicated that oxidative stress is a major factor in the pathogenesis of diabetic complications. This may coincide with the studies of Keany et al., 1993; Lee et al., 1999 which suggested that oxidative stress leads to the accumulation of vascular inflammatory cells, the local production of oxygen derived free radicals and the degradation of endothelium derived relaxing factor and stimulation of P-selectin (a glycoprotein acting as a ligand for leukocyte adhesion) on the surface of endothelial cells. Also, the release of cytokines and chemokines has been demonstrated to promote the recruitment of macrophages from blood stream which in turn promote adhesion, arrest and diapedesis of inflammatory cells through the endothelium with the consequent infiltration of the lipid core (Sengenes et al., 2007).

On the other hand, Ginger administration to diabetic and cholesterol fed rats in this study showed marked but not significant change of blood glucose, total cholesterol, LDL and HDL-cholesterol. This may be consistent with earlier studies (Bordia et al., 1997; Verma et al., 2004) but conflicting with others (Fuhrman et al., 2000;
Al-Amin et al. (2006) which indicated a significant lowering effect of ginger on the previously mentioned parameters. However, this could be attributed to the differences of dose concentration and route of administration followed in these studies. A significant decrease of triglycerides was induced by ginger administration to diabetic cholesterol fed animals in the present work. This effect was also reported in the study of Fuhrman et al. (2000) but in concomitant with a significant decrease of plasma cholesterol. This later study attributed the decrease of cholesterol to the reduction of cellular cholesterol biosynthesis induced by ginger, but didn't explain the way of lowering effect of ginger on triglyceride levels. Therefore, the data of the present study may indicate a role of ginger in suppression of absorption of dietary triglycerides and cholesterol in the intestine and their subsequent excretion.

In the present investigation, ginger lowered lipid peroxidation and elevated plasma total antioxidant capacity and blood reduced glutathione concentration, the finding which strongly confirms the antioxidant properties of ginger reported in previous investigations. Ginger has been reported to have a lowering effect on lipid peroxidation by influencing the enzymatic blood level of superoxide dismutase, catalase, and glutathione peroxidase (Ahmed et al., 2000). It has been also shown that ginger reduces cellular oxidation and scavenge superoxide anion and hydroxyl radicals (Cao et al., 1993, Krishnakantha and Lokesh 1993). Similarly, Siddaraju and Dharmesh (2007) reported that ginger free phenolic and ginger hydrolysed phenolic fractions exhibit free radical scavenging activity. The antioxidative activity of ginger was attributed to scavenging superoxide anion and hydroxyl radicals by some ginger compounds such as gingirols, shogaols and some related phenolic ketone derivatives (Adhikari et al., 2007; Ali et al., 2008).

It could be therefore suggested that antioxidative properties of ginger represent the main way for protecting vascular wall against oxidative stress induced in diabetic cholesterol-fed animals. This effect may work independently of ginger ability to lower increased lipid parameters associated with diabetic complications. In addition to the fact that increased oxidative stress has been shown to be increased in both insulin dependent diabetes and non-insulin dependent (Cederberg et al., 2001) and its main role in vascular disorders, the present investigation confirm the idea that antioxidative activity of ginger plays a central role in vascular protection. However, previous studies pointed to the medicinal potential of ginger against induced oxidative stress (Khaki and Khaki, 2010) and the positive relationship between total phenolic content and antioxidant activities in ginger (Ghasemzadeh et al., 2010).

As revealed by electron microscopy, ginger ameliorated the morphological alterations observed in aortic wall of diabetic cholesterol fed animals in the present study. Although the biochemical data suggest that the antioxidative effect of ginger is principal in attenuation of vascular disease progression, other events reported in previous investigations should be in account with regard to initiation and progression of vascular wall changes. For instance, ginger consumption can result in accumulation of active ingredients within the cells, as well as in the cell plasma membrane, thus affecting cellular enzymes, and plasma membrane receptors. It is also possible that ingredients of ginger such as polyphenols bind to LDL and interfere with the interaction between the lipoprotein hydrophobic domains involved in lipoprotein aggregation (Fuhrman et al., 2000). Ginger decreases blood pressure, which is a risk factor of vascular alterations, via stimulation of muscarinic receptors and blockade of calcium channels and relaxing vascular contraction (Ghayur et al., 2005). Ginger decreases the process of inflammation (Shen et al., 2005) which is implicated in early sages of atherosclerosis. Vasodilator activity was also noted from ginger phenolic constituents 6-, 8- and 10-gingerol and 6-shogaol (Ghayur et al., 2005). Recent investigation (Nogueira de Melo, 2011) showed direct and systemic effects of ginger essential oil on the reduction of leukocyte migration as an important mechanism of the anti-inflammatory action of ginger. This last effect is confirmed by the results of the present investigation where the decrease of adhered monocytes to endothelial cells and intimal macrophages was clearly observed in ginger treated animals.

In conclusion, the results of the present study showed that ginger administration significantly lowered the oxidative stress in diabetic cholesterol-fed rats and decreased the adverse harmful effects on blood vessels. Although the data indicate that the efficacy of ginger as an antioxidant is more potent than its action as a lipid lowering agent, it could be concluded that the antioxidative properties of ginger work in principle with the other benefits of ginger to attenuate and alleviate the initiation and progression of atherosclerosis.

REFERENCES


