The Effect of Ginger (Zingiber officinale) on Oxidative Stress Status in the Small Intestine of Diabetic Rats

Khadem Ansari MH[a], Karimipour M[b], Salami S[a], Shirpoor A[c]

[a]Department of Biochemistry, [b] Department of Anatomy and [c] Department of Physiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, I. R. Iran

Oxidative stress is produced under diabetic conditions and possibly causes various forms of tissue damage in patients with diabetes. The aim of the study was to investigate the effect of ginger on the occurrence of oxidative stress in the small intestine of diabetic rats.

Materials and Methods: Twenty-four male Wistar rats were divided into three groups: control group, nontreated diabetic group, and diabetic group treated with ginger powder as 5% of their daily food. After 6 weeks, lipid peroxidation, protein oxidation, superoxide dismutase (SOD), and catalase levels of the small intestine were measured.

Results: Diabetes caused significant increase of small intestine lipid peroxidation, protein oxidation, and SOD levels and decrease of catalase activity. Lipid peroxidation and protein oxidation were attenuated after consumption of ginger in the diabetic rats, and increased catalase activity.

Conclusions: These findings indicate that ginger, as an oxidant, improves diabetes induced oxidative stress and its complications through prevention of lipid peroxidation and protein oxidation.

Key Words: Diabetes. Ginger, Oxidative stress, Small intestine, Rat

Received: 7.08.2008 **Accepted**: 28.10.2008

Correspondence: Alireza Shirpoor, Department of Physiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

E-mail: ashirpoor@Yahoo.com

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin that frequently results in severe metabolic imbalances and pathological changes in many tissues[1] Dysfunction of the gastrointestinal tract is common among diabetic patients.[2] As many as 75% of patients visiting diabetes clinics report significant gastrointestinal symptoms.[3] The intestinal mucosa is vulnerable to oxidative stress on account of the constant exposure to reactive oxygen species (ROS) generated by several conditions such as ischemia/ reperfusion, inflammatory bowel disease, surgical stress, and diabetes.[4] Increased oxidative stress is important in the development and progression of diabetes and related complications. Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids, and nucleic acids, and eventually cell death. Increased production of high levels of reactive oxygen–free radicals has been linked to glucose oxidation and non-enzymatic glycation of proteins, which contribute to the development of diabetic complications in many tissues.[1]

The cellular antioxidant status determines the susceptibility to oxidative damage and is

usually altered in response to oxidative stress.[5]

The protective effects of exogenously administered antioxidants have been extensively studied in animal models in recent years. Several studies have shown that consumption of antioxidant vitamin and nutrient-rich antioxidants such as ginger decrease diabetic complications and improves the antioxidant system of the body.[6]

Ginger (Zingiber officinale rhizome) is a widely used herbal medicine for the treatment of diseases, including those affecting the digestive tract.[7] The anti–diabetic activity of ginger has been reported in streptozotocin–induced diabetic rats.[8,9]

Considering reports of other studies that document occurrence of oxidative stress in the small intestine of diabetic rats, the present study aimed to investigate the effects of ginger, as an antioxidant, on diabetes-induced alterations enzymatic and oxidative components of the antioxidant defense systems in the small intestine mucosal layer of diabetic rats.

Materials and Methods

Twenty-four male Wistar rats (250–270g) were divided into three groups: control, non-treated diabetic group, and the ginger treated diabetic group that received ginger powder (Adak Co Turkey) as 5% of their daily food[8] for 6 weeks. All experiments on rats were performed in strict compliance with guidelines of the National Institutes of Health Guide for Care and Use of Laboratory Animals (1985).

Diabetes was induced by single intraperitoneal injection of 60 mg/kg body weight streptozotocin (Sigma Company) in 50 mM/l citrate buffer, pH 4.5. The control group was injected intraperitoneally with an equivalent amount of buffer. Blood samples, obtained from tail veins, 48 h after injection of STZ and glucose were analyzed by the glucose oxidase method (Biosystem Kit Barcelona, Spain) for the study, rats with blood glucose higher than 300 mg/dL were considered as diabetics and control and diabetic rats were provided food and water ad libitum throughout the experiment. After 6 weeks, all rats were anesthetized by 10% chloral hydrate (5 ml/kg body weight; Sigma) and blood samples taken from the heart; following this, immediately the abdominal cavity was opened surgically and the whole small intestine removed. The small intestine was segmented and each segment was flushed with chilled 115 g/l KCl solution and mucosa was scraped. A 100 g/l homogenate was prepared in 50 mM/l phosphate buffer, pH 7.44°C in a refrigerated centrifuge (Hermele Germany). The supernatant so obtained was stored at -80°C until it was assayed.[8] Protein content was determined by the method of Bradford 10 using bovine serum albumin as the standard. HbAlc or glycosilated hemoglobin was analyzed by HPLC using automated D–10 Bio Rad hemoglobin analyzers. The extent of protein oxidation was determined by measuring the protein carbonyl content in the supernatant using Cayman kit (Cayman Chemical., MI, USA). In this method, 2, 4–dinitropheylhyrazine (DNPH) reacts with protein carbonyls, forming a Schiff base to produce the corresponding hydrozone, which can be analyzed spectrophotometrically.[11] Catalase activity was determined in the supernatant using the Cayman Kit (Cayman Chemical., MI, USA). The method was based on the reaction enzyme with methanol in the presence of optimal concentration of H_2O_2; the formaldehyde produced was measured by spectrophotometrically with 4-amino–3- hydrazino–5–mercapto-1, 2, 4–triazole (purpald) as the chromogen and measured at 450 nm.[12] Tissue superoxide dismutase (SOD) activity was determined using a Ransod kit (Randox Laboratories, Crumlin, UK). Briefly, the method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-4-iodophenyl-3-4-nitrophenol-5-phenyltetrazolium chloride to form a red formazan dye. SOD activity was measured by the degree of inhibition of the reaction, using absorption at

440 nm. The results were expressed in units per mg of protein. The assay 8–isoprostane (Cayman Chemical., MI, USA) was based on the competition enzyme immunoassay between 8–isoprostane and an 8–Isoprostane-acetylcholin esterase (AChE) conjugate with specific rabbit antiserum; then Ellman's reagent was added. The final product gave a distinct yellow color, absorbed strongly at 412 nm and the amount of 8-isoprostane deter mined spetrophotometrically.[3] All data were expressed as mean±SD. The statistical significance was evaluated by one–way analysis of variance (ANOVA) using SPSS Version 11. 5. A value of P<0.05 was considered significant.

Results

The mean body weight, blood glucose, HbAlc and total protein levels of the non-treated diabetic and ginger-treated groups are given in Table 1, compared with those of the control group.

Table1. Effect of ginger on body weight, blood glucose, HbA_{lc} and total protein in control, diabetic and ginger treated diabetic group

Group	Body weight (g)		Blood glucose (mg/dL)		HbA_{lc}		Total protein small intestine (mg)	
	Initial	6th week	Initial	6th week	Initial	6th week	Initial	6th week
Control	268. 6±9. 8	274. 8±10. 8	146. 5±15	151. 0±12.0	5. 4±0. 1	4. 8±0. 6	NA	7. 0±0. 5
Diabetic	261. 8±24. 0	184. 8±15. 6	362. 3±55.0	604. 4±48.0^{a}	8. 2±3. 1^{a}	29. 9±2. 7^{a}	NA	7. 5±0. 5
Diabetic +ginger	259. 3±17. 8	194. 5±18. 9	345. 2±25.0	380. 3±65.0^{a,b}	6. 2±0. 1^{b}	6. 8±0. 9^{b}	NA	7. 4±0. 4

Data are expressed as mean ± SD; n=8; [a]significant difference compared to control; [b]significant difference compared to diabetic; NA: not applicable

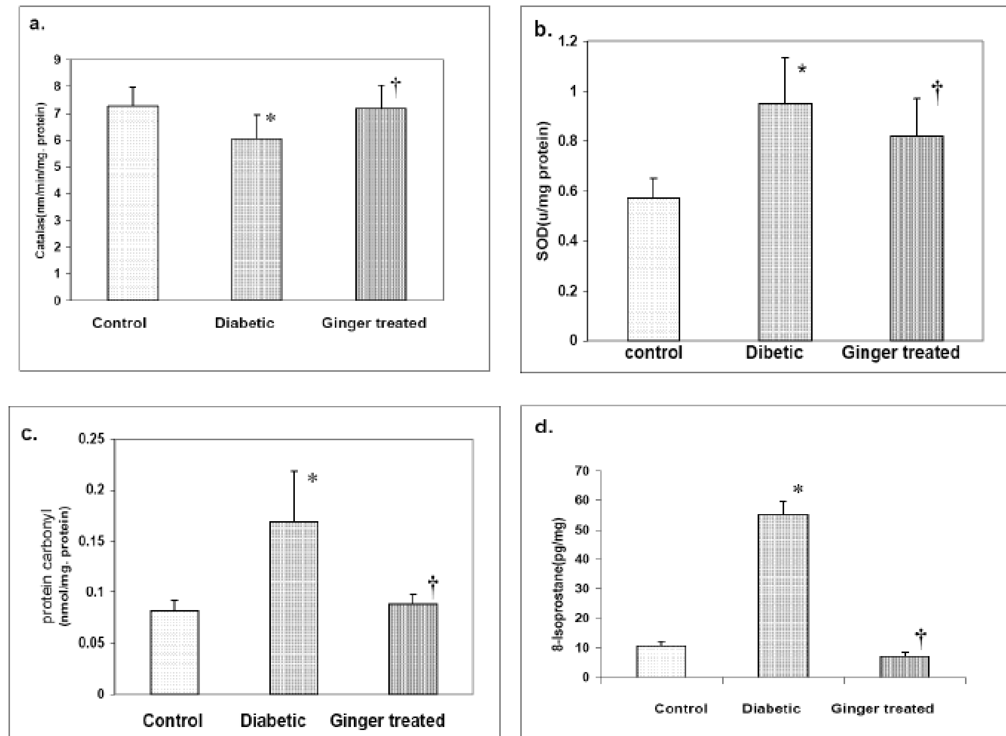
The body weights of rats at the beginning of the study were similar in all groups. At the end of study, after 6 weeks, nontreated diabetic and ginger-treated diabetic rats showed weight loss. However, the body weight loss in ginger-treated rats was lower than that in nontreated diabetic rats.

The blood glucose concentration of the ginger-treated diabetic group at the end of 6 wk decreased significantly compared with that of the nontreated diabetic group (P<0.05). As shown in Table 1, the HbAlc level was significantly higher in nontreated diabetic rats than in control (normal) rats (P<0.001), but it was normalized in the ginger-treated rats. The intestinal levels of total protein did not differ significantly between groups.

Catalase activity in the nontreated diabetic group was significantly decreased compared with that in the other two groups (P<0.05). There was no significant difference between control and the ginger treated groups (Fig. 1a).

The SOD activity in the nontreated diabetic group was significantly higher than in the control group (P<0.001), but, in comparison with ginger treated rats, the difference was not significant. However, the SOD activity in the ginger- treated group was significantly higher than that in the control group (P<0.01) (Fig. 1b). A significant increase in

protein carbonyl content was seen in the non-treated diabetic group compared with the control ($p<0.05$) and ginger- treated group ($P<0.001$), but no significant difference was found between control and ginger-treated rats (Fig.1c). As shown in Fig. 1d, 8- isoprostane level was increased in the diabetic group as compared with the control group ($P<0.001$). In ginger-treated diabetic rats, 8–isoprostane was decreased significantly compared with nontreated diabetic rats ($P<0.001$), whereas in control rats, the difference was not significant.



* Significant difference compared with controls; † Significant difference compared with diabetics

Fig. 1. Biochemical markers of oxidative stress: a. catalase activity b. activity of superoxide dismutase c. protein carbonyl content and d. level of 8 isoprostane

Discussion

In this study, we evaluated the hypothesis that ginger powder prevents hyperglycemia – induced oxidative stress in the small intestine mucosa in STZ–induced type I diabetes in rats. The results proved the occurrence of oxidative damage in the small intestine during the experimental diabetes episode and also revealed that consumption of ginger can improve oxidative stress status with attenuation of lipid peroxidation, protein oxidation, lowering the blood glucose level.

Lipid peroxidation may bring about protein damage and inactivation of membrane-bound enzymes, either through direct attack by free radicals or through chemical modification by its end products, malondialdehyde and 4–hydroxynonenal;[14] It is also known to

decrease the fluidity of the intestinal brush border membrane.[15] Our results showed ginger significantly reduced the extent of lipid peroxidation, which confirmed the findings of other studies.[8]

In this study, the levels of protein–bond carbonyls in the small intestine of non treated diabetic rats were significantly high. Measurement of protein carbonyl is the most widely utilized measure of protein oxidation.[16] It has been proposed that carbonyl stress i.e., the increase in reactive carbonyl compounds derived from oxidative and non–oxidative reactions leads to increased chemical modification of proteins and at a later stage to oxidative stress and tissue damage. A deficit in the detoxification of carbonyl compounds by the enzymes of glyoxalase pathway and aldose reductase is believed to be partly responsible for carbonyl stress and consequent oxidative stress.[17]

The diabetes-induced stimulation of intestinal mucosal growth is believed to be a response to elevated physiological demands. The accompanying increase in the transport of oxidizable compounds such as glucose, amino acids,[18] and lipids,[19] alongwith the increased synthesis of cholesterol and triglycerides[20] and decreased utilization of glucose within the enterocyte,[21] can lead to transient increases in the intracellular concentrations of these compounds. The free radicals generated by autoxidation of these compounds may have been responsible for the elevation in lipid peroxidation and protein oxidation.[4] Our results showed that ginger significantly decreased protein oxidation compared with the nontreated diabetic group. The hypolipidemic effect of ginger has been shown by other investigators[22]. It is likely that the hypochlosterolemic effects of ginger stem from the inhibition of cellular cholesterol synthesis. Attenuation of cholesterol synthesis results in augmentation of LDL receptor activity, which leads to elimination of LDL from plasma.[23] It is well established that elevation of LDL oxidation induces oxidative stress and resultant damage.

8-isoprostane is an oxidative stress marker[24] and its level increases during diabetes. In this study the level of 8-isoprostane was significantly high but reduced levels were found in ginger treated rats.

The HbAlc level in the ginger-treated group was significantly lower than that in the nontreated diabetic group. It has been showed that HbAlc level increases during diabetes[25] and it is a marker which shows the degree of protein glycation.[26] Administration of ginger to diabetic rats significantly decreased the level of glycosylated haemoglobin and this may be due to the decreased level of blood glucose.

To avoid oxidative stress, antioxidant enzymes, such as catalase and SOD, play an important role in reducing oxidative stress.[27] Hyperglycemia–induced oxidative stress plays a key role in the development of diabetes and its complications. There is currently no consensus regarding antioxidant enzyme levels in various organs during the diabetic diseased state. Whereas some studies measuring activities of SOD and catalase in diabetes mellitus show the reductions in levels of these enzymes, others report the increases in the activities of both enzymes with the STZ – induced diabetes. SOD catalyzes the conversion of superoxide radical to H_2O_2. It protects the cell against the toxic effect of superoxide radicals.[28] In the present study, the activity of SOD was significantly high in the small intestine mucosa of the nontreated diabetic rats. The increased SOD activity might be another sign of the increased oxidative stress in the intestinal tissue. The amount of SOD activity in the ginger-treated group was lower than nontreated diabetic group, but the difference was not significant. Ginger might be a scavenger for the free oxygen radicals and it somewhat prevented the elevation of the activities of SOD enzyme in the small intestine of diabetic rats.

Catalase activity decreased significantly in the nontreated diabetic rats compared with that in other groups. Consumption of ginger restored catalase activity to the level similar

to that in control rats. Catalase is one of antioxidative factors involved in elimination of ROS[29] and has a predominant role in controlling the concentration of H_2O_2.[30] However, the increase in catalase activity in the small intestine of diabetic rats has been reported 4 and other investigators reported a lack of changes in the activity of intestinal catalase rats fed diets containing different fat supplement.[31] Such different findings may depend on several factors, such as animal strain, duration of the experiment, severity of diabetes, and assay techniques used to determine of catalase activity. However, other investigators showed decrease of blood catalase activity in diabetic patients.[29]

In conclusion, our results demonstrate that administration of ginger improves oxidative stress induced by type I diabetes by decreasing lipid peroxidation and protein oxidation as free radical generation sources and also by elevating the level of enzymes which implicated in the antioxidant defense system.

It seems that further investigation with bigger sample sizes should be considered to find out the precise mechanism of ginger as an antioxidant and its functional bioactive materials.

References

1. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 2003; 17: 24-38.
2. Zhao J, Forkjaer JB, Drewes AM, Ejskjaer N. Upper gastrointestinal sensory–motor dysfunction in diabetes mellitus. World J Gastroenterol 2006; 12: 2846-57.
3. Folwaczny C, Riepl R, Tschop M, Landgraf R. Gastrointestinal involvement in patients with diabetes mellitus: Part Π (second of two parts).Diagnostic procedure, pharmacological and nonpharmacological therapy. Z Gostroenterol 1999; 37: 803-15.
4. Bhor VM, Raghuram N, Sivakami S. Oxidative damage and altered antioxidant enzyme activities in the small intestine of streptozotocin-induced diabetic rats. Int J Biochem Cell Biol 2004; 6: 89-97.
5. Kakkar R, Kalra J, Mantha SV, Prasad K. Lipid peroxidation and activity of antioxidant enzymes in diabetic rats. Mol Cell Biochem 1995; 51: 113-9.
6. Bianca F, Mira R,Tony H, Raymond C, Michael A. Ginger extract consumption reduces plasma cholesterol, inhibits LDL oxidation and attenuates development of atherosclerosis in atherosclerotic, apolipoprotein E-deficient mice. J Nutr 2000; 130: 1124-31.
7. Borrelli F, Capasso R, Pinto A, Izzo AA. Inhibitory effect of ginger on rat ileal motility in vitro. Life Sc 2004; 74: 2889-96.
8. Taghizadeh A A, Shirpoor A, Farshid A, Saadation R, Rasmi Y, Saboory E. The effect of ginger on diabetic nephropathy, plasma antioxidant capacity and lipid peroxidation in rats. Food Chem 2007; 101: 148-153.
9. 9 Akhani SP, Vishwakarma SL, Goyal RK. Anti–diabetic activity of Zingiber officinale in streptozotocin–induced type I diabetic rats. J Pharm Pharmacol 2004; 56: 101-5.
10. Bradford A, Atkinson J, Fuller N, Rand RP. The effect of Vitamin E on the structure of membrane lipid assemblies. J Lipid Res 2003; 44: 1940-5.
11. Levine RI, Williams JA, Stadtman ER, Shacter E. Carbonyl assay for determination of oxidatively modified proteins. Meth Enzymol 1994; 233: 346-57.
12. Jahansson LH, Borg LAH. A Spectrophotometer method for determination of catalase activity in small tissue samples. Anal Biochem 1988; 174: 331-6.
13. Pradelles P, Grassi J. Enzyme immunoassay of eicosanoids using AchE as label: An alternative to radioimmunoassay. Anal Biochem 1985; 45: 1170-3.
14. Halliwell B, Zhao K, Whiteman M. The gastrointestinal tract: a major site of antioxidant action? Free Radic Res 2000; 33: 819-30.
15. Ohyashiki T, Ohtsuka T, Mohri T. A change in the lipid fluidity of the porcine intestinal brush – border membranes by lipid peroxidation. Studies using pyrene and fluorescent stearic acid derivatives. Biochimica Biophys Acta 1986; 861: 311-8.
16. Dalle-Dnne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta 2003; 329: 23-38.
17. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: A new perspective on an old paradigm. Diabetes 1999; 8: 1-9.
18. Fedorak RN. Adaptation of small intestine membrane transport processes during diabetes mellitus in rats. Can J physiol Pharmacol 1990; 68: 630-5.
19. Staprans I, Rapp JH, Pan XM, Feingold K. The effect of oxidzed lipids in the diet on serum lipo-

protein peroxides in control and diabetic. J Clin Invest 1993; 92: 638-643.
20. Feingold KR, Moser A, Adi S, Soued M, Grunfeld C. Small intestinal fatty acid synthesis is increased in diabetic rats. Endocrinology 1990; 27: 2247-52.
21. Madsen KL, Ariano D, Fedorak RN. Vanadate treatment rapidly improves glucose transport and activates 6-phosphofructo-1-kinase in diabetic rat intestine. Diabetologia 1995; 38: 403-18.
22. Sharma I, Gusain D, Dixit VP. Hypolipidaemic and Antiatherosclerotic Effects of Zingiber officinale in Cholesterol Fed Rabbits. Phytother Res 1996; 10: 517-8.
23. Ness GC, Zhao Z, Lopez D. Inhibitor of cholesterol biosynthesis increase hepatic low density lipoprotein receptor protein degradation. Arch Biochem Biophys 1996; 325: 242-8.
24. Suzaki Y, Ozawa Y, Kobori H. Internal oxidative stress and augmented angiotensinogen are precedent to renal injury in zucker diabetic fatty rats. Int J Biol Sci 2007; 3:40-46.
25. Al–Yassin D, Ibrahim KA. Minor hemoglobin fraction and the level of fasting blood glucose. J Faculty Med Baghdad 1981; 23: 373-80.
26. Kaleem M, Asif M, Ahmed QU, Bano B. Antidiabetic and antioxidant activity of Annona Squamosa extract in STZ- induced diabetic rats. Singapore Med J 2006; 47: 670-675.
27. Sugiura M,Ohshima M,Ogawa K,Yano M. Chronic administration of Satsuma Mandarin fruit (Citrus Unshiu Marc.) improves oxidative stress in STZ – induced diabetic rat liver. Biol Pharm Bull 2006; 29: 588-591.
28. Armagan A,Efkan Uz, Yilmaz Y, Soyupek S,Oksay T, Ozcelik N. Effects of melatonin on lipid peroxidation and antioxidant enzymes in STZ – induced diabetic rat testis. Asian J Androl 2006; 8: 595-600.
29. Cojocaru IM,Cojocaru M,Musuroi C,Botezat M,Lazar L,Druta A. Lipid peroxidation and catalase in diabetes mellitus with and without ischemic stroke. Rom J Intern Med 2004; 42:423-429.
30. Gaetani GF, Ferraris AM, Rolfo M, Mangerni R, Arena S, Kirkman HN. Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes. Blood1996; 87:1569-1599.
31. Giron MD,Salto R,Gonzalez Y,Giron JA,Nieto N,Periago JL,et,al. Modulation of hepatic and intestinal glutathione – S – transferases and other antioxidant enzyme by dietary lipids in STZ – diabetic rats. Chemosphere 1999; 38, 3003-3013.