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Research Article

Modulation of Antioxidant Enzymes and Inflammatory Cytokines: Possible Mechanism of Anti-diabetic Effect of Ginger Extracts

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ABSTRACT: *Zingiber officinale* is used in African traditional medicine to treat diabetes mellitus. Oxidative stress and inflammation are associated with the pathogenesis of diabetes mellitus and its complications. This investigation tested the hypothesis that extracts of *Zingiber officinale* inhibit oxidative stress and inflammation by enhancing antioxidant enzymes and TNF- α activity in STZ-induced diabetic rats. Wistar rats were randomly divided into groups (n=6) receiving different oral treatments consisting of vehicle, aqueous ginger extract (250 and 500 mg/kg), ethanol ginger extract (250 and 500 mg). The effect of *Z. officinale* was assessed in the STZ-induced diabetic rats after 6-week treatment on blood glucose; oxidative stress (using MDA level, SOD, CAT and GSH activities); and inflammation (using TNF- α). Both extracts of *Z. officinale* increased the intracellular activities of SOD, CAT and GSH. The extracts however caused a significant decrease in the MDA and inflammatory TNF- α level. These data indicate that mechanism of antidiabetic effects of ginger may be in part, due to inhibition of oxidative stress and inflammatory activity.

Keywords: Ginger; diabetes; antioxidants; lipid peroxidation, TNF- α

INTRODUCTION

Ginger (*Zingiber officinale*) is a commonly used food spice in many Asian and African countries. Several studies have demonstrated that ginger is endowed with hypoglycaemic properties under normal and diabetic conditions (Ojewole, 2006; Alli *et al*, 2008). Treatment with ginger extract produced a significant reduction in

fasting blood glucose, serum lipids, blood pressure and increasing glucose tolerance in diabetic rats (Akhani *et al*, 2004). Another study conducted by Al-Amin *et al*. (2006) reported that aqueous extract of ginger has hypoglycemic, hypocholesterolemic and hypolipidemic potential at single dose level administered intraperitoneally. The extracts of ginger have also been shown to possess high *in vitro* antioxidant activity against hydroxyl radicals and inhibit lipid peroxidation better than quercetin (Krastanov *et al*, 2007). Previously, we reported that aqueous and ethanol extracts exert strong *in vivo* antioxidant activity against oxidative toxicity from sodium arsenite (Morakinyo *et al*, 2010). In addition, ginger extracts have been reported to produce significant inhibition of carrageenan-induced paw oedema and reduction in the number of acetic acid-induced writhing as well as prostaglandins level in mice and rats (Raji *et al*, 2002; Thomson *et al*, 2002; Penna *et al*, 2003; Grzanna *et al*, 2005).

There is evidence that increasing reactive oxygen species (ROS) is involved in the development and

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progression of diabetes mellitus (Droge, 2002; Maritim *et al*, 2003). Similarly, inflammatory cytokines such as tumor necrosis factor- α (TNF- α) has been implicated in the pathogenesis of diabetes mellitus (Perez – Matute *et al*, 2009). Literature report indicates that TNF- α suppresses insulin signaling in muscles and adipose tissue thereby promoting inflammatory reaction and contributes further to the generation of free radicals (Ueno *et al*, 2002). Based on the fact that extracts of *Z. officinale* are reported to have anti-diabetic potential, it is reasonable to assume that the possible mechanisms of *Z. officinale* therapeutic actions involve due to the extracts ability to maintain endogenous antioxidant levels, suppress oxidative processes and inflammatory activities. However, there is no experimental data on the extracts effects on oxidative stress and inflammation under diabetic condition. Therefore, this study was designed to evaluate the effects of an aqueous and ethanol extracts of *Z. officinale* on oxidative stress using malonaldehyde level, superoxide dismutase, catalase and glutathione activities; and inflammatory activities using tumor necrosis factor- α .

MATERIALS AND METHODS:

Animals

Adult male Wistar rats (8 weeks) weighing 180-200 g were obtained from the Animal House, Indian Institute of Integrative Medicine (IIIM), Jammu. The animals were housed in polycarbonate cages in a room with 12-h day-night cycle, temperature of 24 ± 2 °C and humidity of 45%-64%. Throughout the experimental period, animals were fed a balanced commercial pellet diet (Ashirwad Industries, Chandigarh, India) and water *ad libitum*. The rats were allowed to acclimatize for a period of one week before the commencement of the experiment. All animal handling and experimental protocols adopted in this study complied with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (NIH, 1985).

Drug and Extract Preparation

The *Z. officinale* rhizome (ginger) was purchased from local commercial sources in Lagos, Nigeria. It was identified and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria, by Mr Joseph Ariwaodo and a voucher specimen of the plant was deposited at the herbarium of the Institute (FHI 107935). The plant material was shade dried at room temperature before being pulverized with an electric grinder. The powdered ginger was extracted with distilled water and ethanol using Soxhlet apparatus for

48 h. The extract was concentrated to dryness with rotary evaporator (Helidoph Laborata, Germany) to yield a solid mass of aqueous ginger extract (AGE) and ethanol ginger extract (EGE). The percentage yield was 23.76% (AGE) and 26.51% (EGE). The brownish mass of extracts was always reconstituted in distilled water to the required concentration before administration to animals as test materials.

Induction of experimental diabetes

Experimental animals received a freshly prepared solution of streptozotocin (45 mg/kg) in 0.1 M sodium citrate buffer, pH 4.5, injected intraperitoneally in a volume of 1 ml/100 mg (16). Control rats received 1 ml citrate buffer as vehicle. Five days after streptozotocin administration, rats showing moderate diabetes with glycosuria and hyperglycemia (i.e., blood glucose levels of 250-450 mg/dl) were used for the experiment.

Experimental procedure

A total of 36 rats (30 surviving diabetic rats and 6 normal rats) were used in the experiment. The rats were divided into 6 groups of 6 animals each as follows: group 1, normal untreated rats; group 2, diabetic control rats; group 3, diabetic rats receiving AGE 250 mg/kg body weight; group 4, diabetic rats receiving AGE 500 mg/kg body weight; group 5, diabetic rats receiving EGE 250 mg/kg body weight; and, group 6, diabetic rats receiving EGE 500 mg/kg body weight. The drug and extract administration were given daily by gavage via oral cannula and lasted for a period of 6 weeks. Blood samples were drawn through the retro-orbital sinus at weekly intervals until the end of the 6-week study. At the end of the 6th week, all rats were killed by decapitation after anaesthesia with pentobarbitone sodium (60 mg/kg i.p). Blood was collected into plain sample bottles; allowed to clot and centrifuged at 10,000 rpm for 5 min to obtain serum for the estimation of blood glucose. The liver was dissected out, washed in ice-cold saline, blotted dry, and weighed. The liver homogenate was subsequently prepared and used for oxidative studies.

Biochemical Analyses

All blood glucose measurement were determined by the glucose oxidase method using a commercial kits from Randox Diagnostic Solutions and tumor necrosis factor- α (TNF- α) was assayed using ELISA kits (Cell Sciences) according to the manufacturer's instruction. This assay employed an antibody specific for rat TNF- α coated on a 96-well plate. All the reagents and samples were stored at room temperature before conducting the experiment.

Oxidative Studies

Oxidative analyses of the liver homogenate were carried out using previously described standard methods (Morakinyo *et al*, 2011). Briefly, the most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems, malondialdehyde (MDA) was estimated with the method of Uchiyama and Mihara (Uchiyama *et al*, 1978). The reduced glutathione (GSH) content of the liver homogenate was determined using the method described by Van Dooran *et al*. (1978) while the activity of the SOD enzyme was determined according to the method described by Sun and Zigman (Sun and Zigman, 1978). Catalase (CAT) activity was determined by measuring the exponential disappearance of H_2O_2 at 240nm and expressed in units/mg of protein as described by Aebi (Aebi, 1984). Absorbance was recorded using Shimadzu recording spectrophotometer (UV 160) in all measurements.

Statistical Analysis

Data are expressed as mean \pm standard error of mean (SEM) and analysed using the ANOVA followed by Tukey Kramer post-hoc test. $P < 0.05$ was accepted as significant. All the analyses were carried out using the GraphPad Instat Version 3.05 for Window Vista, GraphPad Software, San Diego California, USA.

RESULTS

Effect of AGE and EGE on fasting blood glucose in STZ-induced diabetic rats

Chronic administration of the AGE and EGE caused significant reduction ($P < 0.05$) in the fasting blood sugar levels of diabetic rats when compared with diabetic controls that were untreated. Table 1 shows that prior to the extract administration, there was no significant difference between the blood glucose levels of the diabetic groups of animals. However, after 6 weeks, the blood glucose levels of the AGE and EGE treated rats were significantly lower than the diabetic control. In contrast, the blood glucose level of the untreated diabetic rat remained elevated throughout the experimental period. The blood glucose level of the normal (healthy) control remained unchanged during the course of the investigation.

Effect of AGE and EGE on lipid peroxidation and oxidative enzymes in STZ-induced diabetic rats

Figure 1 shows the effect of AGE and EGE on lipid peroxidation in diabetic rats. The MDA level was significantly decreased by both AGE and EGE extracts at 250 and 500 mg/kg compared with diabetic untreated rats. The MDA level in diabetic untreated rats was significantly higher than in normal rats. Figure 2 – 3 show the effects of AGE and EGE on antioxidant SOD and CAT activities in diabetic rats. The activities of both enzymes in diabetic untreated rats were significantly lower than the control rats. Activities of both SOD and CAT at all doses of both extracts were significantly higher than the diabetic untreated rats, but significantly lower than the control rats.

Table 1:

Effect of aqueous and ethanol extracts of *Zingiber officinale* on blood glucose level in STZ-induced diabetic rats

	Day -7	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Normal Control	93 \pm 4.56	91 \pm 2.03	90 \pm 2.90	87 \pm 4.08	90 \pm 2.13	92 \pm 3.26	93 \pm 2.62	89 \pm 3.81
Diabetic Untreated	89 \pm 4.08	350 \pm 16.73*	334 \pm 14.57	330 \pm 12.69	337 \pm 10.81	339 \pm 13.45	332 \pm 11.36	338 \pm 10.27
Diabetic + AGE 250	91 \pm 1.82	436 \pm 37.17*	182 \pm 11.25	151 \pm 4.80 [#]	139 \pm 13.64 [#]	132 \pm 7.54 [#]	123 \pm 6.84 [#]	110 \pm 12.37 [#]
Diabetic + AGE 500	88 \pm 2.45	386 \pm 37.44*	154 \pm 15.97 [#]	140 \pm 7.75 [#]	114 \pm 17.5 [#]	125 \pm 10.81 [#]	111 \pm 9.56 [#]	115 \pm 9.72 [#]
Diabetic + EGE 250	87 \pm 2.94	368 \pm 39.70*	160 \pm 13.05 [#]	145 \pm 4.57 [#]	112 \pm 12.13 [#]	134 \pm 9.12 [#]	123 \pm 11.77 [#]	115 \pm 9.72 [#]
Diabetic + EGE 500	90 \pm 3.54	402 \pm 18.98*	152 \pm 6.12 [#]	138 \pm 7.52 [#]	133 \pm 8.70 [#]	128 \pm 7.29 [#]	117 \pm 10.06 [#]	106 \pm 11.64 [#]

Values are expressed as mean \pm SEM ($n = 6$). [#] $P < 0.05$ is significantly different for experimental groups compared with respective diabetic untreated group. * $P < 0.05$, significantly different for diabetic untreated compared with control.

Figure 4 shows the effect of AGE and EGE on GSH activities in diabetic rats. GSH activity in diabetic untreated rats was significantly lower than the control rats. The GSH activities in diabetic rats which received AGE and EGE showed a significant increase than diabetic untreated rats only at the dose of 500 mg/kg. Activities GSH at all doses of both extracts were however significantly lower than the control rats.

Effect of AGE and EGE on TNF- α in STZ-induced diabetic rats

Figure 5 illustrates the effects of AGE and EGE on serum activities of the inflammatory marker, TNF- α in diabetic rats. The TNF- α level in diabetic untreated rats was significantly higher than in normal rats. However, the TNF- α level at all doses of AGE and EGE diabetic rats were significantly lower than the diabetic untreated rats.

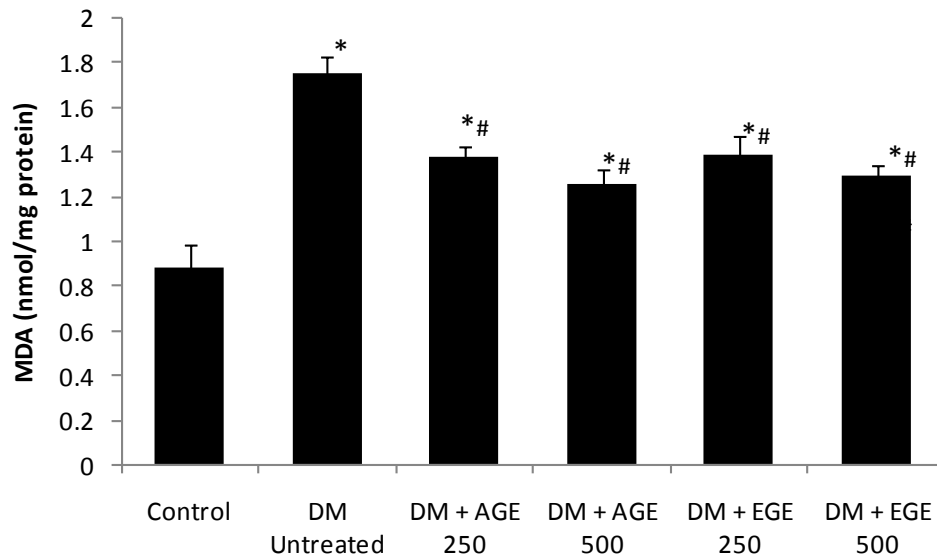


Figure 1:

Effect of aqueous and ethanol extracts of *Zingiber officinale* on MDA level in STZ-induced diabetic rats. #P<0.05, significantly different compared with respective diabetic untreated group; *P<0.05, significantly different compared with control.

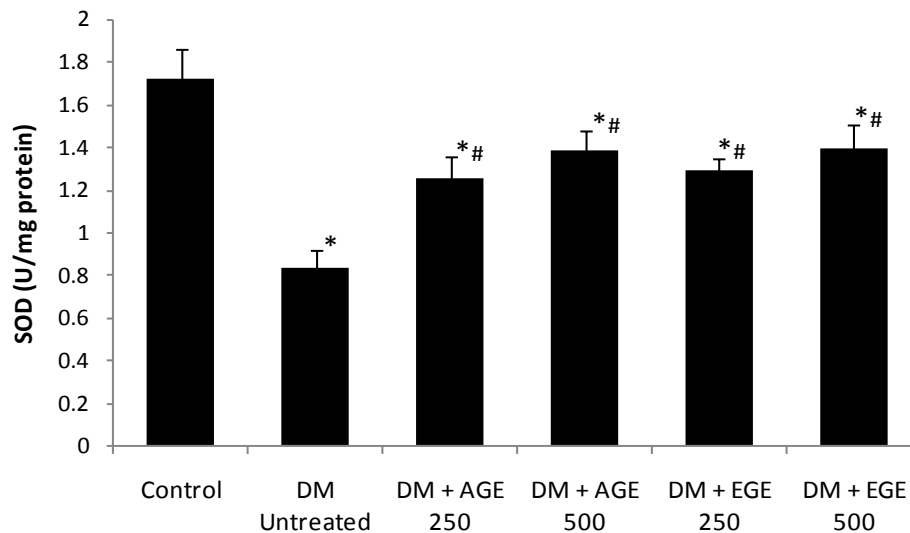


Figure 2:

Effect of aqueous and ethanol extracts of *Zingiber officinale* on SOD activities in STZ-induced diabetic rats. #P<0.05, significantly different compared with respective diabetic untreated group; *P<0.05, significantly different compared with control. One unit of SOD = amount of enzyme needed to dismutate 50% of the superoxide radicals.

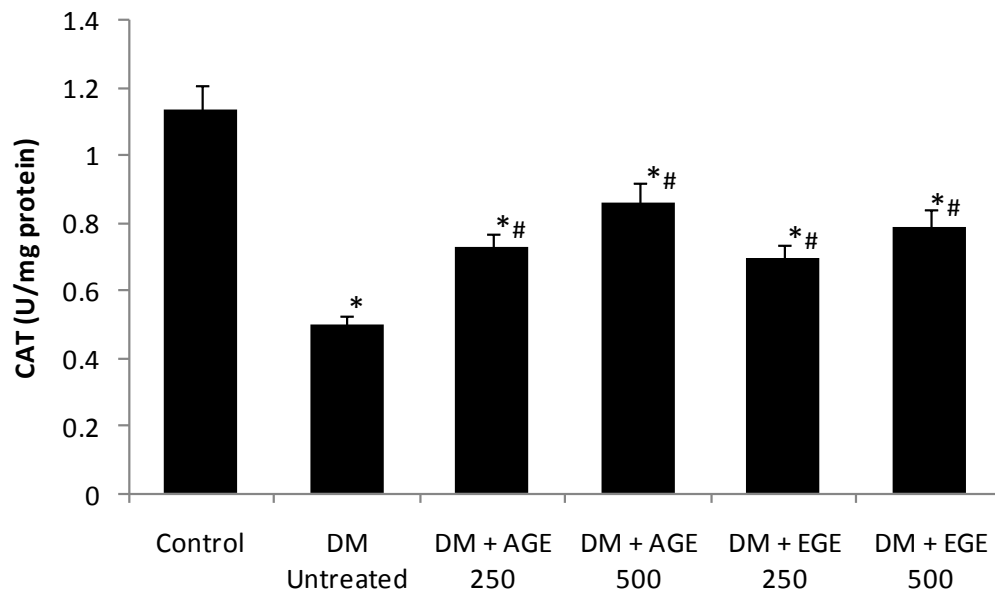


Figure 3: Effect of aqueous and ethanol extracts of *Zingiber officinale* on CAT activities in STZ-induced diabetic rats. [#]P<0.05, significantly different compared with respective diabetic untreated group; *P<0.05, significantly different compared with control. One unit of CAT = amount of enzyme needed to catalyze the decomposition of 1 μ mol of H₂O₂ per minute.

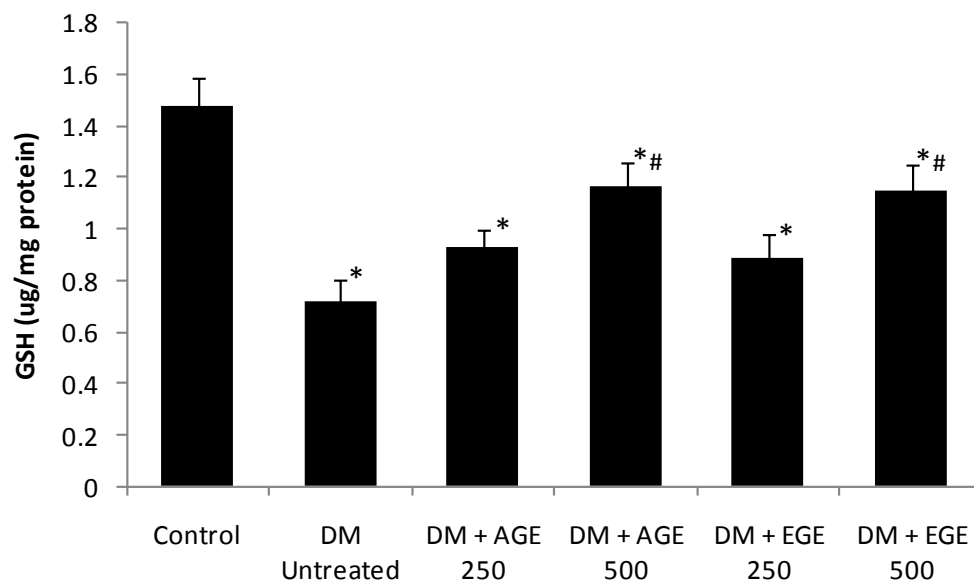


Figure 4: Effect of aqueous and ethanol extracts of *Zingiber officinale* on GSH activities in STZ-induced diabetic rats. [#]P<0.05, significantly different compared with respective diabetic untreated group; *P<0.05, significantly different compared with control.

DISCUSSION

The present work was aimed at assessing the effects of both aqueous and ethanol extracts of *Z. officinale* on oxidative stress and inflammation under diabetic condition. In this study, we found that both extracts of *Z. officinale* significantly reduce the blood glucose level in STZ-induced diabetic rats. It is well

documented that ginger extracts have antihyperglycaemic effects (Akhani *et al*, 2004; Grzanna *et al*, 2005; Al – Amin *et al*, 2006; Alli *et al*, 2008). The present results therefore confirmed that both aqueous and ethanol extracts of ginger enhance glucose utilization since marked reduction were observed in the blood glucose level of diabetic rats.

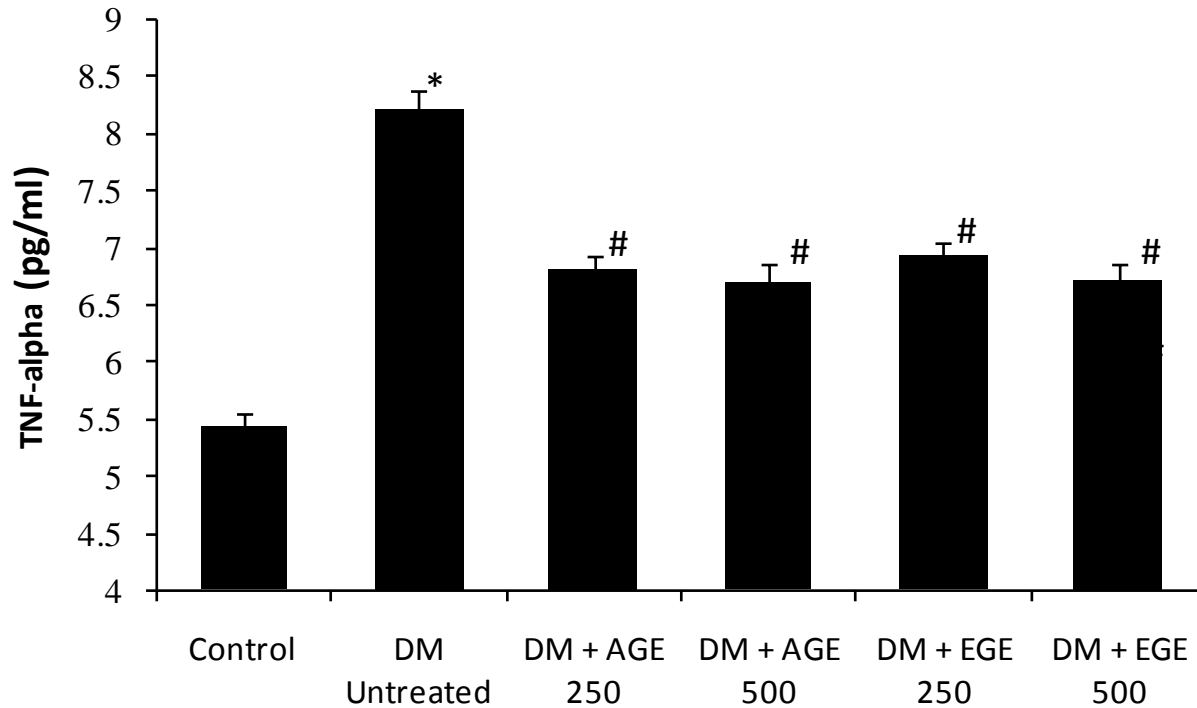


Figure 5: Effect of aqueous and ethanol extracts of *Zingiber officinale* on TNF- α level in STZ-induced diabetic rats. #P<0.05, significantly different compared with respective diabetic untreated group; *P<0.05, significantly different compared with control.

Oxidative stress is widely accepted as a fundamental participant in the development and progression of diabetes mellitus and its complications (Bashan *et al*, 2009). Increasing evidence shows that oxidative stress is elevated under diabetic conditions due to increased production of reactive oxygen species and deficient antioxidant defence (Kakkar *et al*, 1995; Wiernsperger, 2003). Furthermore, there are reports indicating that beta cells are particularly susceptible to reactive species because they are low in free radical quenching (antioxidants) enzymes such as CAT, SOD and GSH (Perez-Matute *et al*, 2009). In this study, we observed an increase in MDA level in STZ diabetic rats indicating increased free radical production and associated lipid peroxidation. However, treatment of diabetic rats with ginger extracts significantly reduced the MDA level. Accordingly, we presumed that treatment of diabetic rats with ginger extracts attenuated the production of free radical and peroxidation of lipids thereby preventing oxidative damage of cellular structures. The results obtained from this study also demonstrated that *Z. officinale* has an ability to increase the intracellular activities of SOD, CAT and GSH enzymes. There was a significant decrease in the SOD, CAT and GSH activities in diabetic-control rats. This is probably indicative of an

insufficient antioxidant defence against free radical mediated damage. Treatment of diabetic rats with ginger extracts however produced increased activities of all these antioxidant enzymes. In view of our findings that ginger extracts up-regulate SOD, CAT and GSH activities in diabetic rat, we suggest that *Z. Officinale* synergistically combats oxidative stress by scavenging free radicals and/or augmenting endogenous antioxidant activities thereby improving diabetic condition.

Cytokines are associated with the pathogenesis of diabetes. One of the three cytokines most often mentioned in descriptions of the pathogenesis of diabetes is tumor necrosis factor- α . TNF- α as a pro-inflammatory cytokine secreted by the macrophages has been implicated in many autoimmune diseases, insulin resistance, diabetes mellitus and cancer (Maechler *et al*, 1999). Because elevated cytokine levels can contribute to accelerated beta-cell apoptosis and death, they are not only part of the primary pathogenesis of type 1 diabetes mellitus but also a potential contributor to the progressive decline in beta-cell function characteristic of type 2 diabetes mellitus. The decreased expression of TNF- α in the diabetic rats receiving ginger extract is consistent with previous reports of Ko and Leung (Ko *et al*, 2010) and Sahebkar

(Sahebkar, 2011). Reduced expression of TNF- α may bring about improved insulin signal transduction in muscles and adipose tissues (Perez-Matute *et al*, 2009) as well as suppressing oxidative stress induced inflammatory reactions (Feinstein *et al*, 1993). Therefore, the antidiabetic effects of ginger extracts possibly involve inhibiting inflammatory activities thereby improving insulin signalling in muscle and adipose tissues. Although a few anti-diabetic agents such as gliclazide and troglitazone have inhibitory effects on TNF- α in diabetic rat (Qiang *et al*, 1998), nevertheless, not many studies have shown the inhibitory effect of natural herbs on TNF- α expression in experimental diabetes to the best of our knowledge. In conclusion, the data from this study indicate that ginger extracts inhibit lipid peroxidation and cytokine TNF- α , however, it enhances endogenous antioxidant activities. These suggest that mechanism of anti-diabetic effects of ginger may be in part, due to inhibition of oxidative stress and inflammatory activity.

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