

## Determination of *in vitro* antidiabetic effects of *Zingiber officinale* Roscoe

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Aqueous extracts of *Zingiber officinale* rhizomes were studied to evaluate their antidiabetic effects on protein glycation and on the diffusion of glucose *in vitro* in the present study. *Zingiber officinale* rhizome aqueous extract were examined at concentrations of 5, 10, 20 and 40 g/L. The antidiabetic effects were found to be dose-dependent. Antidiabetic potential of *Zingiber officinale* was mainly through inhibition of the glucose diffusion and to a limited extent by reducing the glycation. However, further studies are needed to determine *in vitro* effects of therapeutic potential by restraining postprandial glucose absorptions and plasma protein glycations in diabetic subjects.

**Uniterms:** *Zingiber officinale*/pharmacognosy. *Zingiber officinale*/antidiabetic effects/*in vitro* study. Diabetes mellitus. Hyperglycemia. Proteins/glycation. Glucose/diffusion *in vitro*.

Extratos aquosos de rizomas *Zingiber officinale* foram estudados para avaliar os seus efeitos antidiabéticos em glicação de proteínas e sobre a difusão de glicose *in vitro*, no presente estudo. Extratos aquosos de *Zingiber officinale* foram examinados nas concentrações de 5, 10, 20 e 40 g extrato de planta/L. Os efeitos antidiabéticos observados eram dependentes da dose. O potencial antidiabético de *Zingiber officinale* se verificou, principalmente, através da inibição da difusão de glicose e, em menor extensão, através da redução da glicação. Estudos adicionais são necessários para elucidar se efeitos *in vitro* representam potencial terapêutico, restringindo a absorção de glicose pós-prandial e a glicação de proteínas plasmáticas em indivíduos diabéticos.

**Unitermos:** *Zingiber officinale*/farmacognosia. *Zingiber officinale*/efeitos antidiabéticos/estudo *in vitro*. Diabetes mellitus. Hiperglicemia. Proteínas/glicação. Glicose/difusão *in vitro*.

### INTRODUCTION

Incubation of proteins with glucose leads to non-enzymatic glycation and formation of amadori products known as an early glycation product. Non-enzymatic glycation of proteins or Maillard reaction is increased in diabetes mellitus due to hyperglycemia and leads to several complications such as blindness, hepatic and cardiovascular diseases, nerve damage and kidney failure (Hussain *et al.*, 2008, 2011; Chen *et al.*, 2011). Normal glucose homeostasis involves glucose absorption via the gut, production by the liver and utilization by nearly all tissues in the body (Kahn, 1992). Diabetes mellitus (DM), a metabolic disorder caused by insufficient or inefficient

insulin secretory response is characterized by hyperglycemia.

Recently, much attention has been focused on natural and synthetic glycation inhibitors to delay the onset or progression of diabetic complications (Rahman *et al.*, 2009; Peng *et al.*, 2011). The scope for the discovery and development of new anti-diabetic therapies from nature's pharmacy is vast and merits corresponding consideration.

Diet has always been recognized as a corner stone in the management of diabetes mellitus. Spices are known to exert several antidiabetic influences (Srinivasan, 2005). Given the large number of plants reputed to possess antidiabetic properties (Saraswat *et al.*, 2010; Ugwuja *et al.*, 2010; Jafri *et al.*, 2011), only a few have received equitable scientific and medical scrutiny in terms of their antiglycation activities. Another often ignored mechanism of anti-diabetic phytomedicines is the role of viscous polysaccharides that control blood glucose concentration by inhibiting intestinal

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glucose absorption (Hagander *et al.*, 1984; Edwards *et al.*, 1988; Groop *et al.*, 1993; Gallagher *et al.*, 2003).

*Zingiber officinale* (ginger) rhizome is one of the classic examples of an herb used for not only culinary preparations but also for unique therapeutic significance owing to its antioxidant, antimicrobial, anti-inflammatory and chemoprotective potential (Afzal *et al.*, 2001; El-Ghorab *et al.*, 2010; Ghasemzadeh *et al.*, 2010; Rani *et al.*, 2011; Al-Suhaimi *et al.*, 2011; Baliga *et al.*, 2011; Butt, Sultan, 2011; Rehman *et al.*, 2011). Although several studies have mentioned antidiabetic activity of *Zingiber officinale* (Grover *et al.*, 2002; Akhane *et al.*, 2005; Kelble, 2005; Al-Amin *et al.*, 2006; Büyükbacı, Sedef, 2008; Islam, Choi, 2008; Abd-Elraheem *et al.*, 2009; Saraswat *et al.*, 2009; Ogbera *et al.*, 2010; Saraswat *et al.*, 2010; Madkor *et al.*, 2011; Ramudu *et al.*, 2011; Rani *et al.*, 2011), no data is available on protein glycation and glucose absorption inhibitory potential of native *Zingiber officinale* rhizomes at national level. The present study was undertaken to investigate the antidiabetic effects of *Zingiber officinale* in terms of glucose diffusion and glycation inhibition.

## MATERIAL AND METHODS

### Extract preparation

*Zingiber officinale* rhizomes were collected from Botanical garden, University of Agriculture, Faisalabad, Pakistan during March, 2011. The collected rhizomes were homogenized to fine powder and were stored at room temperature. Aqueous extracts of roots were prepared by cold infusion. One gram powdered material was infused for 15 min in 40 mL of boiling distilled water and then filtered, dried under vacuum and stored at -20 °C till further analysis. Antidiabetic activity of *Zingiber officinale* was determined by two methods:

### Glycation inhibition

To study the effect of *Zingiber officinale* on protein glycation process, fifteen combinations of 0.075 M phosphate buffer saline (PBS) with glucose (G<sub>1</sub>: 5.5, G<sub>2</sub>: 25, G<sub>3</sub>: 50 mM), *Z. officinale* (I<sub>1</sub>:5, I<sub>2</sub>:10, I<sub>3</sub>:20, I<sub>4</sub>: 40 g/L as inhibitor) and protein (bovine albumin) were incubated at 37 °C for five weeks (Table I). Samples were analyzed after 3<sup>rd</sup> and 5<sup>th</sup> weeks of incubation. Glucose concentrations were measured and samples were dialyzed to remove free glucose. Free glucose is the major hindrance in estimation of glycation level. Post-dialysis, glucose was again estimated to confirm final glucose levels. Total proteins in all samples after dialysis were determined by biuret

method (Gornall *et al.*, 1949). Thiobarbituric Acid (TBA) colorimetric test was used for the determination of both enzymatic glycation (EG) and non-enzymatic glycation (NEG) as described by Furth (1988).

**TABLE I** - Different combinations used for glycation inhibition study

| Sr.# | Combinations                        | Sr.# | Combinations                        |
|------|-------------------------------------|------|-------------------------------------|
| 1    | P + G <sub>1</sub>                  | 9    | I <sub>2</sub> + P + G <sub>3</sub> |
| 2    | P + G <sub>2</sub>                  | 10   | I <sub>3</sub> + P + G <sub>1</sub> |
| 3    | P + G <sub>3</sub>                  | 11   | I <sub>3</sub> + P + G <sub>2</sub> |
| 4    | I <sub>1</sub> + P + G <sub>1</sub> | 12   | I <sub>3</sub> + P + G <sub>3</sub> |
| 5    | I <sub>1</sub> + P + G <sub>2</sub> | 13   | I <sub>4</sub> + P + G <sub>1</sub> |
| 6    | I <sub>1</sub> + P + G <sub>3</sub> | 14   | I <sub>4</sub> + P + G <sub>2</sub> |
| 7    | I <sub>2</sub> + P + G <sub>1</sub> | 15   | I <sub>4</sub> + P + G <sub>3</sub> |
| 8    | I <sub>2</sub> + P + G <sub>2</sub> |      |                                     |

P: Protein (Bovine albumin), G: Glucose, I: Inhibitor (*Zingiber officinale*), G<sub>1</sub>: 5.5 mM, G<sub>2</sub>: 25 mM, G<sub>3</sub>: 50 mM; I<sub>1</sub>:5 g/L, I<sub>2</sub>:10 g/L, I<sub>3</sub>:20 g/L, I<sub>4</sub>:40 g/L

### Non-enzymatic and enzymatic glycation (collective)

One mL of dialyzed sample (total protein = 10 mg/mL) was used for non-enzymatic and enzymatic glycation. Three test tubes were arranged for each of reduced and non-reduced samples. 0.1 mL of NaBH<sub>4</sub> was added in reduced samples and 0.1 mL of 0.01 N NaOH was added to non-reduced samples. All test tubes were left for 30 minutes at 37 °C. After half an hour, 1 drop of 1N HCl was added in each test tube, followed by addition of 0.5 mL oxalic acid. Tubes were capped and autoclaved for half an hour at 124 °C (115 Lb/inch<sup>2</sup> pressure). Tubes were cooled to room temperature and then placed in ice. In each tube, 0.5 mL of 40% trichloroacetic acid (chilled) was added. Afterwards, samples were centrifuged for 15 minutes at 15000 rpm. Supernatant (1.5 mL) was mixed with 0.5 mL freshly prepared TBA. Later on, the samples were incubated at 37 °C in water bath for 15 minutes and absorbance was determined at 443 nm.

### Enzymatic glycation

For determination of enzymatic glycation (EG), 0.1 mL NaOH (0.01 N) containing 400 molar excess of NaBH<sub>4</sub> was used. After the reduction, the glycation level was determined by the same process as mentioned above. Non-enzymatic glycation(NEG) was determined as follows:

$$\text{NEG} = (\text{NEG} + \text{EG}) - \text{EG}$$

## Glucose diffusion inhibition

The potential of ginger rhizome extracts to inhibit glucose diffusion into the external solution was investigated at set time intervals (Gallagher *et al.*, 2003). In a dialysis tube (6 cm×15 mm), 15 mL of a solution of glucose and NaCl (0.15 M) was introduced and the appearance of glucose in the external solution was measured. The sealed tube was placed in a centrifuge tube containing 45 mL of 0.15 M NaCl. The tube was placed on an orbital shaker at room temperature. Glucose concentrations were measured by glucose oxidase kit method. Incremental areas under the glucose curves (AUC) were calculated by trapezoidal rule.

## Statistical analysis

All data were expressed as mean ± SD and % age ± SD of triplicate measurements. Student's t-test was performed by Statistical Package for the Social Sciences (SPSS Inc. Chicago, IL, USA) software (version 15.0) with level of significance set at  $p < 0.05$ .

## RESULTS

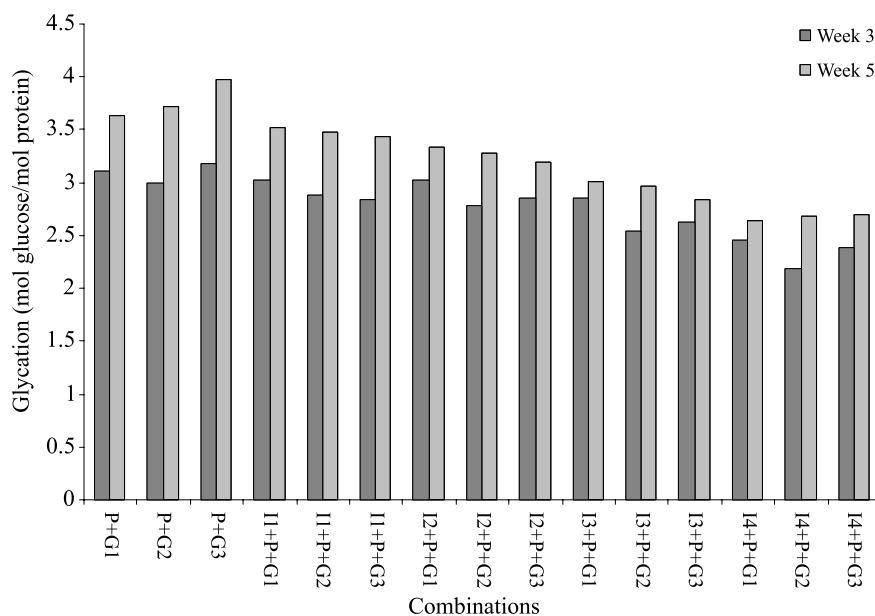
Results of antiglycation potential of *Zingiber officinale* are presented in Figure 1.

For controls, 50 mM ( $G_3$ ) glucose exhibited maximum glycation (3.97 mol glucose/mol protein) after five weeks.  $G_1$  (5.5 mM) and  $G_2$  (25 mM) also showed optimal

glycations (3.64 and 3.72 mol glucose/mol protein). Aqueous extract of 5g/L ginger ( $I_1$ ) reduced glycation levels for  $G_1$  (5.5 mM),  $G_2$  (25 mM) and  $G_3$  (50 mM) as compared to controls and glycation concentrations after 5<sup>th</sup> week were 3.52, 3.48 and 3.44 mol glucose/mol protein, respectively. Likewise, aqueous extract of 10 g/L ( $I_2$ ) ginger lowered glycation levels for  $G_1$ ,  $G_2$  and  $G_3$  up to 3.33, 3.28, 3.19 mol glucose/mol protein, respectively in the final week. Doubling the extract concentrations ( $I_3$ ) as compared to  $I_2$  further decreased glycation levels (for  $G_1$ : 3.01,  $G_2$ : 2.97,  $G_3$ : 2.84 mol glucose/mol protein). Percentage differences in glycation of various combinations from controls reduced significantly. Glycation reduction by  $I_1$  was not significant for any combination after 3<sup>rd</sup> or 5<sup>th</sup> week analysis.  $I_2$  showed significant decrease with  $G_3$  in 5<sup>th</sup> week. Optimum inhibitor concentrations ( $I_3$  and  $I_4$ ) indicated significant decline in glycation measurements after 5<sup>th</sup> week. Most striking observation was maximum glycation inhibition by  $I_4$  (Table II).

A simple model was used to investigate glucose absorption inhibition by *Zingiber officinale*. After 30 h, in the control (without plant extract) glucose movement out of dialysis reached a peak level and glucose concentration in the external solution was 21.20 mmol/L. Different concentrations of *Z. officinale* extracts were used to investigate the dose-dependent effects on glucose diffusion. The plant extracts exhibited a concentration-dependent inhibitory effect on glucose movement (Figure 2).

The effect of variable inhibitor concentrations on



**FIGURE 1** - Effect of aqueous extracts of *Z. officinale* rhizome on glycation levels. Data are mean ± SD for triplicate measurements. P: Protein (Bovine albumin), G: Glucose, I: Inhibitor (*Zingiber officinale*),  $G_1$ : 5.5 mM,  $G_2$ : 25 mM,  $G_3$ : 50 mM;  $I_1$ : 5 g/L,  $I_2$ : 10 g/L,  $I_3$ : 20 g/L,  $I_4$ : 40 g/L

**TABLE II-** Percent decrease in glycation levels at different concentrations of plant extracts

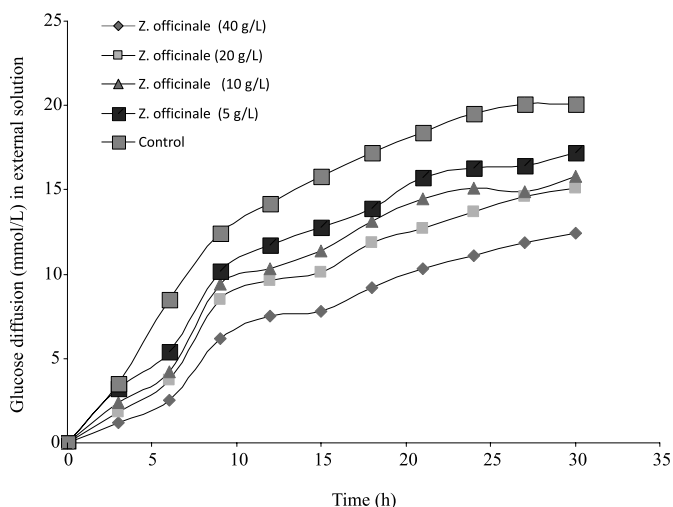
| Combination                          | % Decrease in glycation after 3 weeks <sup>1</sup> | % Decrease in glycation after 5 weeks <sup>1</sup> |
|--------------------------------------|--|--|
| <b>Control (no extract)</b>          |  |  |
| G <sub>1</sub>                       | -  | -  |
| G <sub>2</sub>                       | -  | -  |
| G <sub>3</sub>                       | -  | -  |
| <b>Plant extract (I<sub>1</sub>)</b> |  |  |
| G <sub>1</sub>                       | 2.89 ± 0.08  | 3.30 ± 0.06  |
| G <sub>2</sub>                       | 3.68 ± 0.18  | 6.45 ± 0.02  |
| G <sub>3</sub>                       | 10.69 ± 0.05                                       | 13.35 ± 0.03                                       |
| <b>Plant extract (I<sub>2</sub>)</b> |  |  |
| G <sub>1</sub>                       | 2.57 ± 0.03  | 8.52 ± 0.02  |
| G <sub>2</sub>                       | 7.02 ± 0.06  | 11.83 ± 0.01                                       |
| G <sub>3</sub>                       | 10.38 ± 0.01                                       | 19.65 ± 0.02*                                      |
| <b>Plant extract (I<sub>3</sub>)</b> |  |  |
| G <sub>1</sub>                       | 8.04 ± 0.04  | 17.31 ± 0.01*                                      |
| G <sub>2</sub>                       | 15.5 ± 0.20  | 20.16 ± 0.01*                                      |
| G <sub>3</sub>                       | 17.61 ± 0.01                                       | 28.46 ± 0.03*                                      |
| <b>Plant extract (I<sub>4</sub>)</b> |  |  |
| G <sub>1</sub>                       | 21.22 ± 0.06*                                      | 27.47 ± 0.01*                                      |
| G <sub>2</sub>                       | 26.76 ± 0.03*                                      | 27.69 ± 0.02*                                      |
| G <sub>3</sub>                       | 24.84 ± 0.02*                                      | 31.99 ± 0.01*                                      |

Data are % age ± SD for triplicate measurements.

G: Glucose, I: Inhibitor (*Zingiber officinale*), G<sub>1</sub>: 5.5 mM, G<sub>2</sub>: 25 mM, G<sub>3</sub>: 50 mM; I<sub>1</sub>: 5 g/L, I<sub>2</sub>: 10 g/L, I<sub>3</sub>: 20 g/L, I<sub>4</sub>: 40 g/L

\**p* < 0.05

<sup>1</sup>Percentage decrease in glycation in comparison to control.

**FIGURE 2-** Effect of aqueous extracts of *Z. officinale* on glucose diffusion.

glucose diffusion out of the dialysis tube was also assessed in terms of area under curve (AUC) (Table III).

Extract having 20 and 40 g/L ginger were the most

**TABLE III -** Effect of different concentrations of plant extract on glucose diffusion to external solution

| Sample                      | AUC (mmol/L glucose after 30 h) <sup>1</sup> | % Decrease in Diffusion <sup>2</sup> |
|-----------------------------|--|--------------------------------------|
| <b>Control (no extract)</b> | 416.10 ± 1.52                                | -                                    |
| <b>Plant extract</b>        |  |                                      |
| I <sub>4</sub> (40 g/L)     | 221.70 ± 2.07                                | 46.71 ± *                            |
| I <sub>3</sub> (20 g/L)     | 282.45 ± 0.86                                | 32.1 ± *                             |
| I <sub>2</sub> (10 g/L)     | 309.60 ± 12.36                               | 25.59 ± *                            |
| I <sub>1</sub> (5 g/L)      | 342.60 ± 7.25                                | 17.66 ± *                            |

Data are mean ± SD or % ± SD for triplicate measurements.

*p* < 0.05

<sup>1</sup>AUC: (area under the curve) was calculated using total glucose diffusion during 30 h incubation period as described in the methods section.

<sup>2</sup>Percentage decrease in movement of glucose into the external solution in comparison to control.

effective inhibitors of glucose movement in the model system. In the presence of 20 g/L inhibitor, glucose diffusion was significantly decreased and external glucose concentrations were 15.1 mmol/L after 30 h. This represents 32.1% decrease in total glucose diffusion compared to control (*p* < 0.05). Similarly, 40 g/L ginger revealed more than 45% decrease in the glucose movement (*p* < 0.05) compared to control with a mean external glucose concentration of 12.4 mmol/L after 30 h. Extracts containing I<sub>1</sub> and I<sub>2</sub> inhibitor concentrations were least effective. These extracts demonstrated a mean external glucose concentration of 17.20 and 15.80 mmol/L after 30 h. The external glucose concentrations after 30 h were greater at 5 g/L compared to 40 g/L (17.2 vs 12.4 mmol/L respectively, *p* < 0.05). Aqueous ginger extracts demonstrated significant inhibitory effects on glucose movement into external solution across dialysis membrane compared to control. For all the test samples, the overall rates of glucose movement into external solution were lower than that of control. The results underline the importance of *Zingiber officinale* against the diabetic conditions mainly through inhibition of the glucose absorption and to a lesser extent by its antiglycating potential. The therapeutic use of nutrients needs further exploration for the prevention or delay of diabetic complications.

## DISCUSSION

Nonenzymatic glycation of proteins or Maillard reaction is increased in diabetes mellitus due to hyperglycemia (Hussain *et al.*, 2008; Cárdenas-León *et al.*, 2009; Hussain *et al.*, 2010; Hussain *et al.*, 2011; Schalkwijk, Miyata, 2012). Numerous synthetic and natural com-

pounds with different glycation inhibition mechanisms are reviewed in literature. However, owing to the side effects of synthetic molecules, all future expectations rely on natural antiglycation compounds (Peng *et al.*, 2011).

Ginger can lower the blood glucose, improve activities of mitochondrial enzymes and could be used as a nephro-protective supplement particularly to reverse diabetic complications (Saraswat *et al.*, 2010; Ramudu *et al.*, 2011). The potential effects of *Zingiber officinale* in terms of protein glycation and glucose diffusion inhibition were evaluated in the present study.

Thiobarbituric colorimetric assay was used to establish the concentration and time dependent inhibition of protein glycation by ginger. Four different concentrations of aqueous extracts of ginger rhizomes were used to determine the time-dependent changes in protein glycation levels after three and five weeks of incubation at 37 °C in the presence and absence of plant extracts. Ginger extract concentrations of I<sub>3</sub> (20 g/L) and I<sub>4</sub> (40 g/L) showed significant percent reduction ( $28.46 \pm 0.03$  -  $31.99 \pm 0.01$ ) in NEG. Hence the extracts showed a dose-dependent inhibition of protein glycation. Previously, 63% glycation inhibition by ginger at the concentration of 1.0 mg/mL was reported (Saraswat *et al.*, 2009).

Reduction in glycation can be justified by the fact that non-enzymatic glycation is the covalent binding of reducing sugars to  $\alpha$ - or  $\epsilon$ - amino groups on protein. This glycation lowering activity can be linked to the presence of various phenolic components in ginger rhizomes. The pungent fractions of ginger, namely gingerols, shogaols, paradols, and volatile constituents like sesquiterpenes and monoterpenes, are mainly attributed to the health-enhancing perspectives of ginger (Al-Amin *et al.*, 2006; Ojewole, 2006; Islam, Choi, 2008; Butt, Sultan, 2011). The NEG inhibitory activity of ginger depends, at least in part, on its ability to trap reactive carbonyl intermediates in NEG/AGE formation, thereby inhibiting the chemical modification of proteins. In terms of preventing protein and sugar cross-links, the aqueous extracts of ginger showed 30-80% reductions (Saraswat *et al.*, 2009).

The above-mentioned inferences confirm the ability of *Z. officinale* extracts to lower the protein glycation in an empirical *in vitro* manner by TBA assay. Other chemical methods and *in vivo* models might not be able to produce similar reductions in protein glycation process by these extracts.

Incubation period also affected glycation process. The percentage inhibitions in NEG after third week became significant when incubations were prolonged to five weeks indicating decline in potential glycation with time. In recent years, much interest has been focused on the role

of viscous polysaccharides in the treatment of diabetes mellitus. The action of the polysaccharides in reducing postprandial hyperglycemia is thought to be related to their viscosity. Viscous polysaccharides may delay glucose absorption probably by impairing the access of luminal contents to the absorptive epithelium (Hagander *et al.*, 1984; Edwards *et al.*, 1988; Groop *et al.*, 1993).

Ginger extracts also prevented or reduced glucose movement and evaluated percent decrease predicted the action of the plant polysaccharides on hyperglycemia *in vivo*. High concentration extracts (20, 40 g/L) showed greater percent decrease (32%-46.7%) in glucose movement. The results suggested that the part of the antihyperglycemic actions of ginger may be by decreasing glucose absorption *in vivo*. The main mechanism concerning the role of dietary fiber in lowering postprandial serum glucose is the viscosity of different dietary fibers in hampering diffusion of glucose and postponing absorption and digestion of carbohydrates as these bind glucose and resultantly decrease the concentration of available glucose in the small intestine (Ou *et al.*, 2001). Gallagher *et al.* (2003) studied the ability of different plants to inhibit glucose diffusion. They reported that agrimony and avocado represented the greatest inhibitory effect on glucose diffusion (more than 60%). Mushrooms, coriander, eucalyptus, juniper, lucerne and mistletoe decreased significantly (ranged 6%-48%). While elder and nettle extracts did not significantly decrease glucose diffusion. Contrary to these findings, Büyükbacı and Sedef (2008) observed that the overall rates of glucose movement into external solution were higher than control for ten aqueous herbal tea extracts. None of the samples demonstrated significant inhibitory effects on glucose movement into external solution across dialysis membrane compared to the control. Further studies are required to elucidate *in vitro* effects regarding postprandial glucose absorptions and for improving glycemic control in diabetic subjects.

In conclusion, results of the present study suggest that *Z. officinale* rhizomes with antiglycating and anti-diabetic/hypoglycemic properties might provide a viable approach, either food based or pharmacological, in the treatment of diabetic complications.

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