Comparative analysis of antiglycation potential of vegetables aqueous and methanolic extracts

Munawar Iqbal¹, Muhammad W. Ashraf² and Muhammad Bilal²,3,*

¹National Centre of Excellence in Physical Chemistry, University of Peshawar, Peshawar-25120, Pakistan
²Department of Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan
³School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China

Abstract

Present study was conducted to appraise the antiglycation potential of vegetables aqueous and methanolic extracts. The extracts (sweet potato, turnip and methi) were studied in combination with different glucose levels. Eight different combinations of each extract were selected and incubated for five weeks at 37°C. Human normal blood plasma was used as a protein source. Glycation was estimated by Thiobarbituric Acid (TBA) technique and it was observed that aqueous and methanol extracts of sweet potato and turnip did not show non-enzymatic glycation inhibition, whereas aqueous extract of methi inhibited non-enzymatic glycation which was higher at 5th week of incubation and methanolic extract showed maximum activity after 3rd week of incubation.

Keywords: Vegetables extracts
Antiglycation
Thiobarbituric acid
Non-enzymatic glycation

INTRODUCTION

Non-enzymatic glycation (glycosylation) is a multistage condensation reaction between reducing sugar and amino group (mainly Lys and Arg) of different proteins (Stoynev et al., 2004). These glycated proteins rearrange and give rise to a stable product that degrades into a variety of compounds, more reactive than the sugars from which they are derived (Wautier and Schmidt, 2004). These propagators again form yellow-brown, often fluorescent, irreversible compounds, called Advanced Glycation End-Products (AGEs) or Maillard products (Hatfield, 2007) which lead to the progression of atherosclerosis, Alzheimer's disease (Stoppa et al., 2006) and diabetes mellitus (Forbes et al., 2004). These disorders are characterized by hyperglycemia and many chronic complications in blood vessels, eyes, skin, nerves and kidneys (Ahmad and Ahmed, 2006). Non-enzymatic glycosylation (Glycation) process may contribute to formation of discoloration, off-flavors and decreased nutritional value (Nursten, 2005).

A state hyperglycemia exists in diabetes, where non-enzymatic glycation, lipid oxidation and oxidation of protein occur and results in accumulation of AGEs and has been linked to pathogenic complication (Lalla et al., 2001). When sugar molecules react with lipid and protein under the controlled action of enzymes then this process is called glycosylation; during the process of glycation functions of some biomolecules is disturbed, whereas in glycosylation this process occur on specific molecules and at specific sites required for proper functioning (Ahmed & Furth, 1992).
Plants are used as an excellent medicinal source. Traditional uses of plants for diabetic patients and ancient literature on the plant research performed from several decades have shown property of anti-diabetes. Active natural compounds and their crude extract have shown clinical and anti-diabetic activity. These plants include: *Allium cepa*, *Aloe vera*, *Momordica charantia*, *Pterocarpus marsupium*, *Tinospora cordifolia* and *Trigonella foenum graecum* (Grover et al., 2002). Present research was conducted to evaluate the inhibitory effect of sweet potato, turnip and methi aqueous and methanol extracts against advanced glycation end products formation.

**MATERIAL AND METHODS**

Sweet potato (*Ipomoea batatas*), turnip (*Brassica rapa rapa*) and methi (*Trigonella foenum-graecum*) were collected from local market, Faisalabad, Pakistan. To study the inhibitory effects on glycation or glycation inhibition in vitro, eight combinations of each inhibitor were made with plasma (protein source) and glucose and were placed at 37°C for five weeks (Zhang and Swaan, 1999). Samples were drawn after 1st, 2nd, 3rd, 4th and 5th week of incubation and tested for glycation inhibition. Different glucose and inhibitor concentration were tested at 37°C.

**Plasma collection:** Normal plasma was collected from civil hospital, Faisalabad. The plasma was pooled from the blood of healthy person. To carry out the whole study, total 200 mL plasma was used.

**Extraction:** The potato, turnip and methi were air dried and 15 g of each was dissolved in 150 mL of distilled water and methanol and stirred for 48 h at room temperature. Then, samples were filtered, evaporated and filtrate was stored in glass bottles for their use as aqueous extracts.

**Estimation of browning and total protein contents:** The sample collected at different time intervals were subjected to browning and protein estimation. For browning 0.1 mL and 4 mL distilled water was taken, mixed and absorbance was measured at 370 nm (CE Cecil 7200) and total protein contents were measured by Biuret method (Zhang et al., 1999).

**Measurement of Glycation level:** The glycation level was measured by TBA method (Furth, 1988). For glycation measurement, glycated plasma samples were dia lyzed against distilled water for 24 h with constant stirring at room temperature to remove the free glucose. The glycation level was measured by TBA (Thio-barbituric acid) method. For non-enzymatic glycation, 0.5 mL plasma and 0.1 mL of 0.01 N NaOH was taken and test tube was kept at 37°C for 30 min. Then, one drop of 1 N HCl and 0.25 mL of oxalic acid (2N) was added. Then tubes were autoclaved for 15 min, cooled and 40% chilled TCA (0.5 mL) was added. Samples were centrifuged for 15 min at 13,000 rpm. After centrifugation, 0.5 mL of TBA (2N) was added in 1 mL of supernatant, incubated at 37 ºC for 15 min and absorbance was measured at 443 nm. For enzymatic glycation estimation, the absorbance was also measured at 443 nm.

**RESULTS AND DISCUSSION**

The browning value of plasma with buffer and glucose was 0.233 at 1st week, which decreased to 0.196 at 2nd week and 0.184 in 3rd week without extracts. In the 4th week, the browning value again increased and reached to 0.229 and decreased after 5th week of incubation (0.221). In the presence of inhibitor (sweet potato aqueous extracts), the browning value increased and values were recorded to 0.227, 0.250, 0.395, 0.501 and 0.379 after 1st, 2nd, 3rd, 4th and 5th week of incubation, respectively (Fig. 1A). In case of combination of turnip aqueous extracts, plasma, glucose and buffer, the browning values were recorded to be 0.582, 0.307, 0.368, 0.353 and 0.385 after 1st, 2nd, 3rd, 4th and 5th week of incubation, respectively (Fig. 1B). For methi aqueous extracts showed minimum browning value which were found to be 0.196, 0.225, 0.171, 0.157 and 0.204 after 1st, 2nd, 3rd, 4th and 5th week of incubation, respectively (Fig. 1C). Overall, browning value in aqueous extracts were found in following order; turnip > sweet potato > methi. In case of methanolic extracts of sweet potato, the browning values were 0.836, 0.926, 0.403, 0.897 and 0.768 after 1st, 2nd, 3rd, 4th and 5th week of incubation, respectively (Fig. 2A), whereas turnip methanolic extracts showed browning of 0.565, 0.635, 0.478, 0.673 and 0.512 after 1st, 2nd, 3rd, 4th and 5th week of incubation, respectively (Fig. 2B). The methi methanolic extracts again showed lower browning values as compared to sweet potato and turnip (Fig. 2C).

The glycation level in control extract was recorded to be 0.365 and 0.280 mole/mole in 1st and 2nd week of incubation. In case of sweet potato aqueous extracts (inhibitor), glucose and buffer combination, the glycation values increased form 1st to 3rd week and reached to 0.646 and then decreased slightly in subsequent weeks (Fig. 3A). In case of turnip aqueous extracts, the glycation level was 0.572 mole/mole at 4th week and 0.908 mole/mole after 3rd week of incubation (Fig. 3B). The glycation level of 0.266 and 0.240 mole/mole was recorded after 4th and 1st week of incubation (Fig. 3C). Overall, the order of glycation level in aqueous extract was observed as; turnip > sweet potato > methi. In case of methanolic extracts, the glycation level was found to be 0.727 and 0.437 mole/mole after 2nd week and 3rd week of incubation (Fig. 4A). The glycation level was also recorded maximum and comparable with sweet potato methanolic extracts after 2nd week of incubation (Fig. 4B). In case of methi methanolic extracts, the glycation level was 0.285 and 0.203 mole/mole after 2nd and 3rd week of incubation, respectively (Fig. 4C). Overall, the order of glycation value of methanolic extract was found as; sweet potato > turnip > methi. Results revealed that methi aqueous and methanolic extracts are more effective in anti-glycation activity as compared to sweet potato and turnip.

These finding supported the previous findings that plants have anti-glycation active compounds e.g. Marles and Farnsworth, (1995) demonstrated that the hypoglycaemic activity of *Trigonella foenum-graecum* is because of its active components. Chemical compounds isolated from *Trigonella foenum-graecum* include alkaloids, saponins and steroids etc. Zia et al. (2001) also revealed that *Trigonella foenum-graecum* can be used as an herbal medicine. Seeds of *Trigonella foenum-graecum* are known for their antidiabetic, tonic carminative effects. The oral route of administration for methanolic extract
produced hypoglycaemic effect at the dose of 1 g/ kg body weight. In aqueous and methanolic extract, presence of hypoglycaemic activity is due to active compounds which are polar in nature. Bierhaus et al. (1998) explored that products mostly derived from carbohydrate starts accumulating in tissue proteins at high rate with increasing age and in diabetes which are products of oxidation and glycation reaction. Anwar and Meki (2003) studied that in streptozotocin induced-diabetic rat; impaired status of antioxidant can effectively be normalized by garlic oil.

Diabetes complications as neuropathy, nephropathy and retinopathy can be delayed by garlic oil. Sheikh et al. (2004) observed that diabetes leads to protein glycation which in turn affects biochemical activity and structure of proteins. Garlic has a significant effect in decreasing or inhibiting the albumin glycation reaction and decreasing diabetic complications. Hwang et al. (2005) suggested that glycation-derived free radicals formation and formation of AGEs in vitro is inhibited by aged garlic extract (AGE). Aged garlic key component S-Allylcysteine is a potent antioxidant which inhibits the formation of AGEs. Ou et al. (2007) suggest that four garlic derived organosulfur compounds (diallyl disulfide, DADS; diallyl sulfide, DAS; N-acetylcysteine, NAC; S-ethylcysteine, SEC) are potent agents against glycation and oxidation for protecting LDL and they may benefit in preventing complications of diabetes mellitus and cardiovascular disease patients.

CONCLUSION

The anti-glycation activity of aqueous and methanolic extracts of sweet potato, turnip and methi was studied. It was found that methanolic extract of methi showed maximum inhibition of non-enzymatic glycation at 3rd week, whereas aqueous extract showed inhibitory effect after 5th week of incubation. The sweet potato and turnip did not show anti-glycation potential and may be useful for the reduction of diabetes and cardiovascular complications.

REFERENCES


