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Comparative Analysis of Antiglycation Capacity of Aqueous and Methanolic Extracts of Vegetables

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Abstract

Glycation is a reaction between amino group of blood proteins and reducing sugars in vitro conditions which are involved in a number of pathologies and disease states including Alzheimer?s and diabetes. Equal concentration of different inhibitor extracts (sweet potato, turnip and methi) and glucose were used. Eight combinations of each extract were made and all these were placed at 37oC for five weeks incubation. Human normal plasma was used as a protein source. Glycation was analyzed by Thiobarbituric acid (TBA) technique which results that aqueous and methanol extracts of sweet potato and turnip showed no inhibition of non-enzymatic glycation but act as activator of reaction while aqueous extract of methi showed maximum inhibition of non-enzymatic glycation in 5th week of incubation and for methanol extract inhibition was maximum in 3rd week of incubation. In all extracts of three vegetables, extracts of methi were more effective against non-enzymatic glycation. These findings suggest that in future methi can be used for lowering glucose level in the body as it is efficient in lowering the glycation level in different conditions when level of glucose is high.

Index terms—

1 Introduction

on-enzymatic glycation (glycosylation) is a multistage condensation reaction starting between reducing sugar and amino group (mainly in Lys and Arg) of different proteins (Stoynev et al., 2004) there are twofold meaning of non-enzymatic glycation: on one hand, early glycation product measurement which give estimation of glucose exposure and previous metabolic control of the subject; while on the other hand, intermediate and the late glycation reaction products measurement (Lapolla et al., 2005) ending up with complex heterocyclic compound formation called advanced glycation end products (AGEs) (Stoynev et al., 2004) lead in progression of atherosclerosis, Alzheimer's (Stoppa et al., 2006) and particularly in diabetes mellitus which is a endocrine disorder (Forbes et al., 2004) characterized by hyperglycemia and many chronic complications affecting the blood vessels, eyes, skin, nerves, and kidneys (Ahmad and Ahmed, 2006). Non-enzymatic glycosylation (Glycation) process, also known as Maillard reaction, (Hatfield, 2007) may contribute to formation of discoloration, off-flavors and decreased nutritional value (Nursten, 2005).

The intermediate appearance leads to the Amadori compound formation (an aldosylamine; aldose initial reaction with amino groups results in the formation of Schiff's base, which slowly rearrange itself for the production of 1-amino-1-deoxyketose, an aldosylamine) occurs in glycation early stages, however in late stage of glycation, irreversible formation of advanced glycation end products (AGEs) occur after a repeated reactions complex cascade as condensation, cyclization, dehydration, fragmentation and oxidation (Kikuchi et al., 2003). A state hyperglycemia found in diabetes, where non-enzymatic glycation, lipid oxidation and oxidation of protein occur. As a result, accumulation of advanced glycation end product (AGEs) in diabetic subject's tissues and the plasma. Accumulation of this AGE has been linked to pathogenic complication the development in diabetes (Lalla et al., 2001).

2 II.

3 Materials and Methods

Research work was planned to find out the inhibition of glycation with natural inhibitor i.e. Sweet potato, turnip and methi.

4 a) Selection of Conditions and concentrations

To study the inhibitory effects on glycation or glycation inhibition invitro, eight combinations of each inhibitor were made with plasma and glucose, and were placed at 37°C for five weeks (Zhang and Swaan, 1999). Plasma was used as a protein source. Samples were drawn after 1st, 2nd, 3rd, 4th and 5th week of incubation to perform the experiments for glycation and glycation inhibition. Along with temperature (37°C) different concentrations of glucose and inhibitor were used.

5 b) Estimation of Browning

Browning was estimated by taking absorbance at 370nm using spectrophotometer. After every week one sample was drawn and took 0.1 ml from it. Rest of the sample was kept in refrigerator at -20°C. In 0.1 ml of sample 4ml of distilled water was added and 4.1 ml volume was obtained. Then absorbance was taken at 370nm by spectrophotometer. Blank samples will be run with each condition of glucose and inhibitor concentration. c) Total proteins estimation (g/dL)

Total proteins in all samples before and after dialysis were determined by Biuret method using Biuret reagent (Gornall et al., 1949). 1ml of Biuret reagent was added in blank, standard and all samples tubes. Placed the tubes at 37°C for 15 minutes and reading was taken at 540nm. The standard curve was made with the half of absorbance of standard solution.

6 III.

7 Dialysis

Glycated plasma samples were dialyzed against dist. H2O for twenty-four hours with constant stirring at room temperature to remove the free glucose by using dialyzing membrane.

8 a) Measurement of Glycation level

The glycation level was measured by TBA method **??**Furth, 1988). b) Thiobarbituric acid (TBA) colorimetric technique TBA technique **??**Furth, 1988) was used for the determination of both enzymatic and non-enzymatic glycation. The standard curve was made by using fructose standard solution.

IV.

9 Results and Discussion

10 a) Estimation of Browning

Combination of plasma with buffer and glucose showed maximum browning (0.233) at 1 st week of incubation while value of browning decreases to (0.196) at 2 nd week. In 3 rd week of incubation was at its minimum value (0.184). In the 4 th week it increases to (0.229) and in the 5 th week browning was (0.221). In the next combination of plasma with inhibitor sweet potato, glucose and buffer gives maximum level of browning. Plasma with buffer and glucose combination showed browning (0.168) at 1 st week of incubation while value of browning moves to maximum which was (0.177) at 2 nd week. In 3 rd week, incubation was at its minimum value (0.148). In the 4 th week it increases to (0.158) and in the 5 th week browning was (0.152). Combination of plasma with Turnip as inhibitor, glucose and buffer in the next showed maximum browning in the 1 st week of incubation which was (0.582) then it move to its lowest value of combination which was (0.307) in the 2 nd week. In the 3 rd week it gets (0.368) then in the 4 th week it was (0.353) and it shows 2 nd highest value of browning in the 5 th week which was (0.385). Combination of plasma with buffer and glucose showed maximum browning (0.286) at 1 st week of incubation while value of browning moves to minimum of its combination which was (0.253) at 2 nd week. In 3 rd week of incubation it starts increasing gradually which was (0.259). In the 4 th week it gets (0.265) and in the 5 th week of incubation browning was (0.276). In the next combination of plasma with Methi as inhibitor, glucose and buffer showed browning in the 1 st week of incubation which was (0.196) then it move to its maximum value of combination which was (0.225) in the 2 nd week. Combination of plasma with buffer and glucose showed browning (0.155) at 1 st week of incubation while value of browning increases to (0.161) at 2 nd week. In 3 rd week of incubation browning moves to maximum of combination which was (0.191). In the next combination of plasma with Turnip as inhibitor, glucose and buffer showed browning in the 1 st week of incubation (0.565) then the value of browning increases to (0.635) in the 2 nd week. In the 3 rd week it was lowest of combination (0.478) then in the 4 th week it showed highest browning of its combination (0.673) and value of browning in the 5 th week was (0.512). Incubation of plasma with glucose and buffer showed maximum glycation level at 1 st week of decreased glycation level (.280 mole/mole) recorded in 2 nd week. Combination

of plasma, sweet potato as inhibitor, glucose and buffer showed highest value of glycation (.646 mole/mole) at 3 rd week of incubation which gradually decreases in coming two weeks. In case of glycation inhibition, inhibitor act as activator of glycation reaction as it showed minimum value (.394 mole/mole) in 1 st week of incubation. Incubation of plasma with glucose and buffer showed maximum glycation level at 4 th week of combination which was (.274 mole/mole) while decreased in glycation level (.169 mole/mole) recorded in 1 st week. Combination of plasma, turnip as inhibitor, glucose and buffer showed highest value of glycation (.908 mole/mole) at 3 rd week of incubation which decreases in coming week. In case of glycation inhibition, inhibitor act as activator of glycation reaction as it showed minimum value (.572 mole/mole) in 4 th week of incubation. Incubation of plasma with glucose and buffer showed maximum glycation level at 5 th week of combination which was (.342 mole/mole) while decreased glycation level (.274 mole/mole) recorded in 4 th week of incubation. Combination of plasma, methi as inhibitor, glucose and buffer showed highest value of glycation (.266 mole/mole) at 4 th week of incubation with a gradual increase from 1 st week.

V.

11 Conclusion

In case of non-enzymatic glycation, methanol extract of methi showed maximum inhibition of glycation in 3 rd week of incubation as compare to aqueous extract which showed minimum value of inhibition in 5 th week of incubation. On thorough study it is concluded that methanol extract of methi is more effective in glycation inhibition. Reference Références Referencias

12 VI. Discussion

1

Figure 1: Figure 1 :

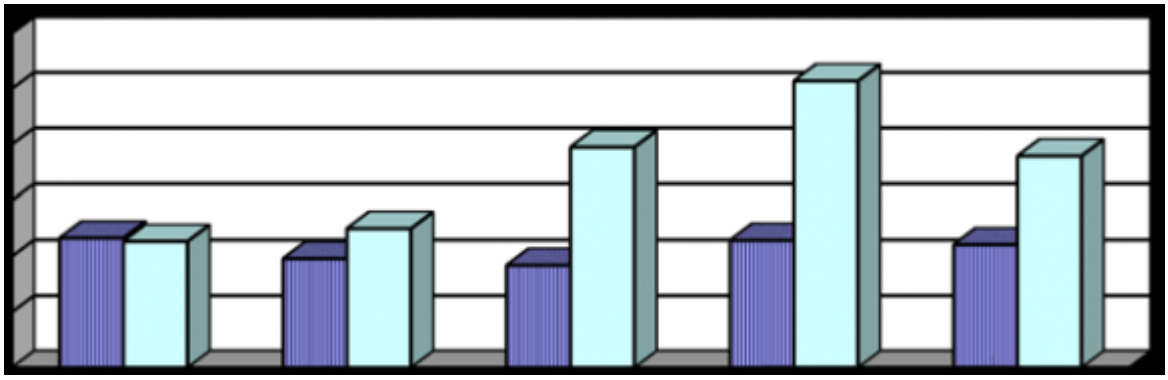

Figure 2:

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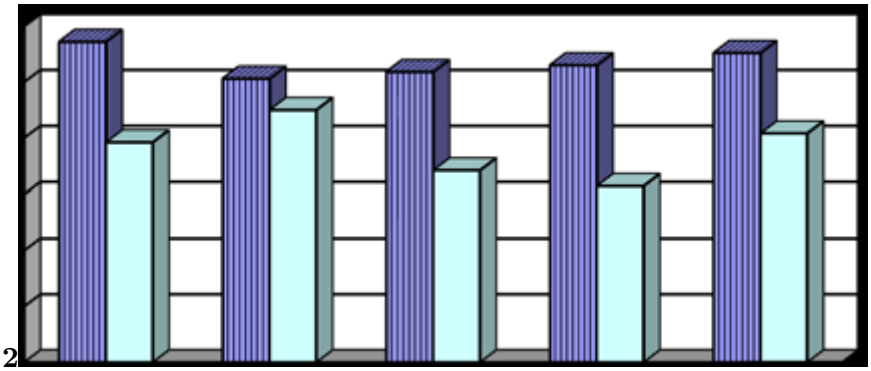

2

Figure 3: Figure 2 :

1. Ahmad and Ahmed (2006) demonstrated that diabetes mellitus is a common endocrine disorder characterized by hyperglycemia and long-term complications affecting the eyes, nerves, blood vessels, skin, and kidneys.
2.

Figure 4:

[Makita et al. ()] 'Advance glycosylation end products in patients with diabetic nephropathy'. Z Makita , S Radoff , E J Rayfield , Z Yang , E Skolnik , V Delaney , E A Friedman , A Cerami , H Vlassara . *Journal of Diabetes Complications* 1999. 325 p. .

[Gillery ()] 'Advanced glycation end products (AGEs), free radicals and diabetes'. P Gillery . *Journal of Social Biology* 2001. 4 p. .

[Marles and Farnsworth ()] 'Antidiabetic plants and their active constituents'. R J Marles , N R Farnsworth . *Phytomedicine* 1995. 2 p. .

[Zhang and Swaan ()] 'Determination of Membrane protein Glycation in Diabetic Tissue'. E Y Zhang , P W Swaan . *AAPS Pharmaceutical Sciences* 1999. 1 p. .

[Gornall et al. ()] 'Determuination of serum proteins buy means of biuret reactions'. A G Gornall , C S Bardwill , M M David . *J. Biol. Chem* 1949. 177 p. .

[Grover et al. ()] 'Evaluation of antihyperglycemic and hypoglycemic effect of Trigonella foenum-graecum Linn, Ocimum sanctum Linn and Pterocarpus marsupium Linn in normal and alloxanized diabetic rats'. J K Grover , S Yadav , V Vats . *Journal of Ethnopharmacol* 2002. 79 p. .

[Zia et al. ()] 'Evaluation of the oral hypoglycaemic effect of Trigonella foenum-graecum L. (methi) in normal mice'. T Zia , S N Hasnain , S K Hasan . *Journal of Ethnopharmacology* 2001. 75 p. .

[Bernargi ()] *explained that glycation is a non-enzymatic process in which proteins react with reducing sugar molecules and thereby impair the function and change the characteristics of the proteins. Glycation is involved in diabetes and aging where the accumulation of glycation products causes side effects*, Morten A Bernargi , E . 2006.

[Stoppa ()] *found that non-enzymatic glycation is implicated in the development of various diseases such as Alzheimer's and diabetes mellitus. An increase in the generation of reactive oxygen species can occur by non-enzymatic glycation and glucose autoxidation*, Stoppa . 2006.

[Kikuchi et al. ()] 'Glycationa sweet tempter for neuronal death'. S Kikuchi , K Shinpo , M Takeuchi , S Yamagishi , Z Makita , N Sasaki , K Tashiro . *Brain Research Reviews* 2003. 3 p. .

[Lapolla ()] *non-enzymatic glycation has a twofold meaning: on one hand, measurement of early glycation products can estimate the extent of exposure to glucose and the subject's previous metabolic control; on the other hand, measurement of intermediate and late products of the glycation reaction is a precious instrument in verifying the relationship between glycation products and tissue modifications*, Joney J Lapolla . 2005.

[Lalla et al. ()] 'Receptors of advanced glycation end products inflammation and accelerated periodontal disease in diabetes'. E Lalla , I R Lasmaster , A M Schmidt . *Ann. Periodontal* 2001. 6 (1) p. .

[Nursten ()] *said that Maillard reactions are a complex set of reactions, typically occurring between carbonyl compounds and amino groups originating from proteins, peptides or amino acids. Maillard reactions may lead to formation of offflavours, decreased nutritional value and discolouration*, Nursten . 2005.

[Stoynev and Ahmed ()] *said that nonenzymatic glycosylation (glycation) of proteins is a multistage chemical process starting as a condensation reaction between reducing sugars and primary amino groups (mainly from the side chains of Lis and Arg) and ending up with formation of complex heterocyclic compounds called*, S Stoynev , D Ahmed . 2004. (advanced glycation end products (AGEs)

[Hatfield ()] *stated that Glycation (nonenzymatic glycosylation) processes, also known as the Maillard reactions, are a series of reactions between carbohydrates and free amino groups of proteins*, Hatfield . 2007.

[Thomas and Morey ()] *studied prolonged hyperglycemia, dyslipidemia and oxidative stress in diabetes result in the production and accumulation of AGEs*, E Thomas , C Morey . 2005.