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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.

I. Introduction

Non-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).

II. Materials and Methods

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

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 37ºC 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 (37ºC) different concentrations of glucose and inhibitor were used.

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 -20ºC. 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.

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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.

III. 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.

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. Results and Discussion

a) Estimation of Browning

Combination of plasma with buffer and glucose showed maximum browning (0.233) at 1st week of incubation while value of browning decreases to (0.196) at 2nd week. In 3rd week of incubation was at its minimum value (0.184). In the 4th week it increases to (0.229) and in the 5th 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 1st week of incubation while value of browning moves to maximum which was (0.177) at 2nd week. In 3rd week, incubation was at its minimum value (0.148). In the 4th week it increases to (0.158) and in the 5th week browning was (0.152). Combination of plasma with Turnip as inhibitor, glucose and buffer in the next showed maximum browning in the 1st week of incubation which was (0.582) then it move to its lowest value of combination which was (0.307) in the 2nd week. In the 3rd week it gets (0.368) then in the 4th week it was (0.353) and it shows 2nd highest value of browning in the 5th week which was (0.385). Combination of plasma with buffer and glucose showed maximum browning (0.286) at 1st week of incubation while value of browning moves to minimum of its combination which was (0.253) at 2nd week. In 3rd week of incubation it starts increasing gradually which was (0.259).

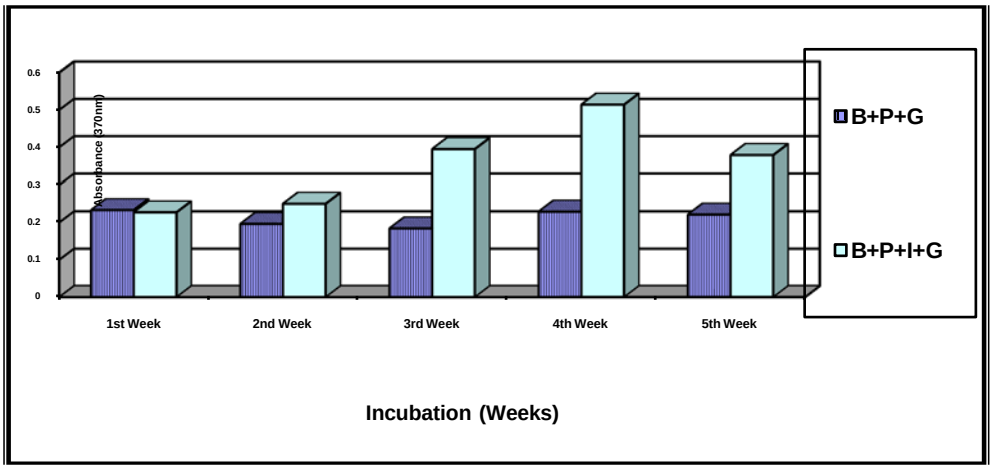

Figure 1: Determination of Browning by the Aqueous Extract of Sweet Potato (S P) at 37°C

In the 4th week it gets (0.265) and in the 5th week of incubation browning was (0.276). In the next combination of plasma with Methi as inhibitor, glucose and buffer showed browning in the 1st week of incubation which was (0.196) then it move to its maximum value of combination which was (0.225) in the 2nd week. Combination of plasma with buffer and glucose showed browning (0.155) at 1st week of incubation while value of browning increases to (0.161) at 2nd week. In 3rd 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 1st week of incubation (0.565) then the value of browning increases to (0.635) in the 2nd week. In the 3rd week it was lowest of combination (0.478) then in the 4th week it showed highest browning of its combination (0.673) and value of browning in the 5th week was (0.512).

b) Thiobarbituric Acid Test

Incubation of plasma with glucose and buffer showed maximum glycation level at 1st week of

combination which was (.365 mole/mole) while decreased glycation level (.280 mole/mole) recorded in 2nd week. Combination of plasma, sweet potato as inhibitor, glucose and buffer showed highest value of glycation (.646 mole/mole) at 3rd 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 1st week of incubation. Incubation of plasma with glucose and buffer showed maximum glycation level at 4th week of combination which was (.274 mole/mole) while decreased in glycation level (.169 mole/mole) recorded in 1st week. Combination of plasma, turnip as inhibitor, glucose and buffer showed highest value of glycation (.908 mole/mole) at 3rd 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 4th week of incubation.

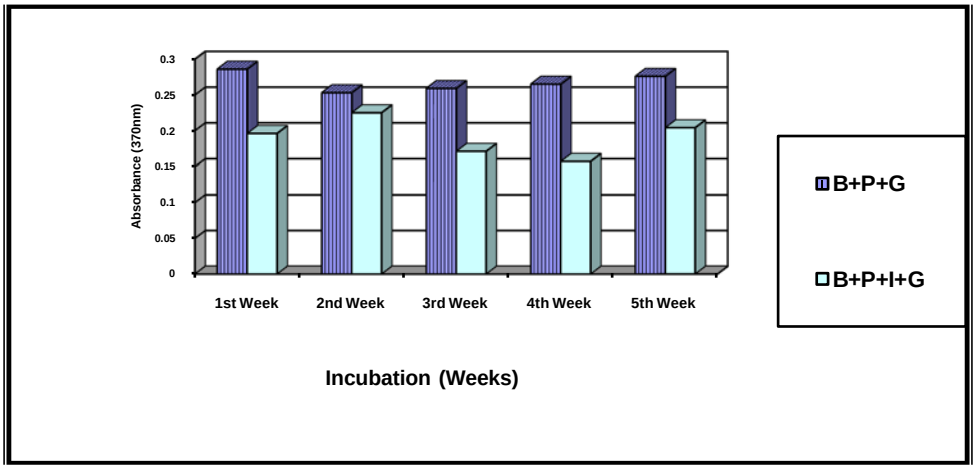

Figure 2 : Determination of Browning by the Aqueous Extract of Methi (M) at 37°C

Incubation of plasma with glucose and buffer showed maximum glycation level at 5th week of combination which was (.342 mole/mole) while decreased glycation level (.274 mole/mole) recorded in 4th week of incubation. Combination of plasma, methi as inhibitor, glucose and buffer showed highest value of glycation (.266 mole/mole) at 4th week of incubation with a gradual increase from 1st week.

V. Conclusion

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

VI. Discussion

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. Marles and Farnsworth, (1995) demonstrated that the hypoglycaemic activity of *Trigonella foenum-graecum* is because of its active components chemical nature of. Chemical compounds isolated from *Trigonella foenum-graecum* include alkaloids, saponins and steroids etc. Zia *et al.* (2001) said that *Trigonella foenum-graecum* (Fenugreek) (Leguminosae) is also being 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 because of active compounds which are polar in nature.

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