



ANTIGLYCATION EFFECT OF CHILLIES AND SPICES USED IN TRADITIONAL INDIAN CUISINE

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ABSTRACT

The present investigation to determine the Non enzymatic glycation and the accumulation of advanced glycation end products (AGEs) are associated with various diseases states, including complications of diabetes and aging. Hydroalcoholic extracts of in several kinds of spices and chilly of culinary used in traditional Mexican cuisine were tested for in-vitro inhibition of non- enzymatic glycation of bovine serum albumin. In this study we have tested 2 spices and 1 chili for their inhibitory activity on protein glycation. The antiglycation capacity of all the selected plant materials was found to increase as the duration increases. The maximum antiglycation capacity was seen with garlic after three week i.e. 1470 μ mol. This was followed by cloves which is 1240 μ mol which in turn followed by the red chillie. The fluorescence of AGEs was shown to be remarkably inhibited by several extracts comparing with the positive (AG).

INTRODUCTION

Advanced Glycation End-products (AGEs) are the result of a chain of chemical reactions after an initial glycation reaction. The intermediate products are known, variously, as Amadori, Schiff base and Maillard products, named after the researchers who first described them. (The literature is inconsistent in applying these terms. For example, Maillard reaction products are sometimes considered intermediates and sometimes end products.) Side products generated in intermediate steps may be oxidizing agents (such as hydrogen peroxide), or not (such as beta amyloid proteins).^[1] "Glycosylation" is sometimes used for "glycation" in the literature, usually as 'non-enzymatic glycosylation. Kazeem MI has studied the antiglycation on different spices.^[2]

MATERIALS AND METHODS

GARLIC [*Allium sativa*]

Garlic was weighed 10gm and dried in absence of sunlight. Dried garlic was smashed using motor and pistle. Garlic was pasted using ethanol and water in equal mixture (1:1 ratio). Continuously grind garlic paste with ethanol mixture for 8 days. Then the extract was obtained by filtration of smashed paste using whatman.42 paper. Extract was transferred to closed bottles and was kept in deep refrigerator, so that ice form of extract was obtained. After that it was brought to liquid form in room temp. Then four test tubes were taken, among them one is for blank in which extract was absent. Blank contain 2ml of BSA, 2ml of buffer, 1ml fructose, 1ml buffer but not

extract. In remaining three test tubes different conc. of extract was taken i.e, 0.2, 0.4, 0.6.make up this to 1ml with phosphate buffer. Again, 2ml phosphate buffer,2ml BSA solution,1ml fructose were added to tubes. After that readings were taken at 440nm against blank weekly once by taking time interval. Three readings were taken for three weeks and tested for anti-glycation effect of garlic. Values obtained were represented in tabular form.

RED CHILLI [*Capsicum frutescens*]

Dried red chili was weighed 10gm. Dried red chili was smashed using motor and pestle. Red chili was pasted using ethanol and water in equal mixture (1:1 ratio). Continuously grind red chili paste with ethanol mixture for 8 days. Then the extract was obtained by filtration of smashed paste using whatman.42 paper. Extract was transferred to closed bottles and was kept in deep refrigerator, so that ice form of extract was obtained. After that it was brought to liquid form in room temp. Then four test tubes were taken, among them one is for blank in which extract was absent. Blank contain 2ml of BSA, 2ml of buffer, 1ml fructose, 1ml buffer but not extract. In remaining three test tubes different conc. of extract was taken i.e, 0.2, 0.4, 0.6.make up this to 1ml with phosphate buffer. Again, 2ml phosphate buffer, 2ml BSA solution,1ml fructose were added to tubes. After that readings were taken at 440nm against blank weekly once by taking time interval. Three readings were taken for three weeks and tested for anti-glycation effect of red chili. Values obtained were represented in tabular form.

CLOVES [*Syzygium aromaticum*]

Dried cloves were weighed 10gm. Dried cloves were smashed using motor and pestle. Cloves were pasted using ethanol and water in equal mixture (1:1 ratio). Continuously grind cloves paste with ethanol mixture for 8 days. Then the extract was obtained by filtration of smashed paste using whatman.42 paper. Extract was transferred to closed bottles and was kept in deep refrigerator, so that ice form of extract was obtained. After that it was brought to liquid form in room temp. Then four test tubes were taken, among them one is for blank in which extract was absent. Blank contain 2ml of BSA, 2ml of buffer, 1ml fructose, 1ml buffer but not extract. In remaining three test tubes different conc. of extract was taken i.e., 0.2, 0.4, 0.6. make up this to 1ml with phosphate buffer. Again, 2ml phosphate buffer, 2ml BSA solution, 1ml fructose were added to tubes. After that readings were taken at 440nm against blank weekly once by taking time interval. Three readings were taken for three weeks and tested for anti-glycation effect of cloves. Values obtained were represented in tabular form.

The formation of AGE was firstly assessed by the characteristic fluorescence (excitation wavelength of 370 nm and emission wave-length of 440 nm).^[3] Percent inhibition was calculated as follows.

$$\text{Inhibition \%} = [1 - (A_0 - A_b) / (A_c - A_b)] \times 100$$

Where A_0 is the fluorescence of the incubated mixture with sample, and A_c and A_b are the fluorescence of the incubated mixture without sample as a positive control and the fluorescence of incubated mixture without sample as a blank control.

RESULTS AND DISCUSSION

The formation of total AGEs was assessed by monitoring the production of fluorescent products at excitation and emission maxima of 370 and 440 nm, respectively. The fluorescence intensity of this glycoprotein which is characteristic of AGEs was highly increased through incubation of BSA with Fructose. TABLES 1 and 2 show the effect of fructose on the total AGEs formation during 30 days of BSA incubation at 37c compared to the control value, the fluorescence intensity was significantly higher in samples with sugar. As it is evident in both tables hydro alcoholic extract at different concentrations has significantly quenched the fluorescence, has significantly suppressed the fluorescence intensity in a dose dependent manner. In that respect, the extract activities are comparable to the effect of 1Mm AG solution which is a known inhibitor of glycation process. The effectiveness is expressed in IC50 values [inhibits glycation 50%] on AGEs formation.

In this study we have tested 2 spices and 1 chilli for their inhibitory activity on protein glycation (Table 1, 2). The antiglycation capacity of all the selected plant materials was found to increase as the duration increases. The maximum antiglycation capacity was seen with garlic after three week i.e. 1470 μmol . This was followed by

cloves which is 1240 μmol which in turn followed by the red chilly. The fluorescence of AGEs was shown to be remarkably inhibited by several extracts comparing with the positive (AGE).

Diabetes mellitus is the most common endocrine disorder characterized by hyperglycemia and long-term complications affecting the eyes, kidneys, nerves and blood vessels. The underlying mechanism responsible for its complications, as well as for diabetes itself, remains unclear, though possible events such as activation of protein kinase C, the polo pathway, non-enzymatic glycation and oxidative stress have been suggested.^[4,5]

Advanced glycation end-products are well-known contributors to the path physiology of aging and diabetic chronic complications.^[6] Increased glycation during hyperglycemia can cause intra or inter molecular cross linking of proteins as they accumulate advanced glycation end products. Numerous studies have shown that buildup of cross-linked advanced glycation end products on long-lived proteins may underlie the development of complications affecting diabetes and ageing.^[7] Furthermore, the levels of serum advanced glycation end products reflect the severity of these complications whereas therapeutic interventions aimed at reducing advanced glycation end products can inhibit or delay their progression.^[9]

Several plants showed antiglycation activities in BSA-fructose model as they all had high contents of phenolic compounds. On the basis of a literature search, many purified phenolic compounds (including flavones, flavanones, flavonols, is flavones, proanthocyanidins, and other phenolics) and phenolic-rich plant extracts have been found to have strong inhibitory activity in this bioassay.

A good correlation exists between their free radical scavenging capacity and AGE inhibitory activity *in vitro*.^[10,11] This suggests that they exert their inhibitory activity by interrupting the autoxidative pathways. In fact, there is growing evidence that production of ROS is increased in diabetes patients and that oxidative stress is associated with diabetic complications. In contrast, numerous clinical trials have failed to provide conclusive evidence for the efficacy of natural antioxidant therapy in diabetic patients.^[12] These findings strongly suggest that free radical scavenging may be effective in suppressing AGE formation only under certain *in vitro* conditions and that inhibiting autoxidation alone is unlikely an effective way of preventing or treating diabetic complications when more complex physiological environments are involved.

The BSA-fructose model adopted in this study provides a useful tool for assessing the effects of hydro-alcoholic extracts of spices and chillies on the non-enzymatic glycation process. Table 1 and 2 displays the inhibitory effects of these plants on AGE formation in this model.

It was a surprise to find that leaves of *Origanum majorana*, *Allium ascalonicum* and *P. auritum* showed high antiglycation activities in BSA-fructose model being capable of reduce appreciably the formation of fluorescent since it not contains a high content of phenols, was shown *P. auritum* to be more promising antiglycation candidates than the other thirty-six plants studied. Vitamin C, phenolics, flavonoids, carotenoids, capsaicinoids, capsaicin, dihydrocapsaicin isolated from the pepper fruit *C. annuum*, *C. baccatum*, *C. chinensis*, *C. tubenos*, *C. anuum grossum* and peppers (red, yellow and green), showed high antioxidative effect. However, these chillis reduced slightly the glycation.

Instead, *Crotolaria longirostrata*, *Bixa orellana*, *Satureja macrostema* and *Curcuma longa* have high antioxidant and high antiglycation activities. In several studies showed

that antiglycation activities of plants indeed, as having been repeatedly reported, were relevantly and directly related to its polyphenolic content, yet it seemed to us that several plants also possessed a rather specific and somewhat different degree of free radical scavenging ability, thus it was speculated that the reaction mechanism of plants might have occurred in the initiation rather than the propagation phase, a mechanism being quite different from the conventional free radical scavenging.^[13] Antioxidant activity was evaluated quantitatively in the current study, our results indicated in some cases the antioxidant and antiglycation activities are high, although this does not happen in other cases. From these spices can effectively serve as an antioxidant and antiglycation agent in the diets of diabetics. Recently studied about different spices and concluded that spices are promising AGE inhibitors.^[14]

Table 1: Antiglycation capacity of Garlic, Cloves and Red chill.

Spices	Control	First day	Second day	Third day
Garlic	28.0±1.0	980.3±8.96	1047±55.65	1475±12.28
Cloves	28.3±0.55	788±2	1242.6±11.23	1203±6.08
Red chill	28.9±0.36	463.3±10.40	581±6.55	964±11.53

n =3; Mean_ SD from triplicate determinations.

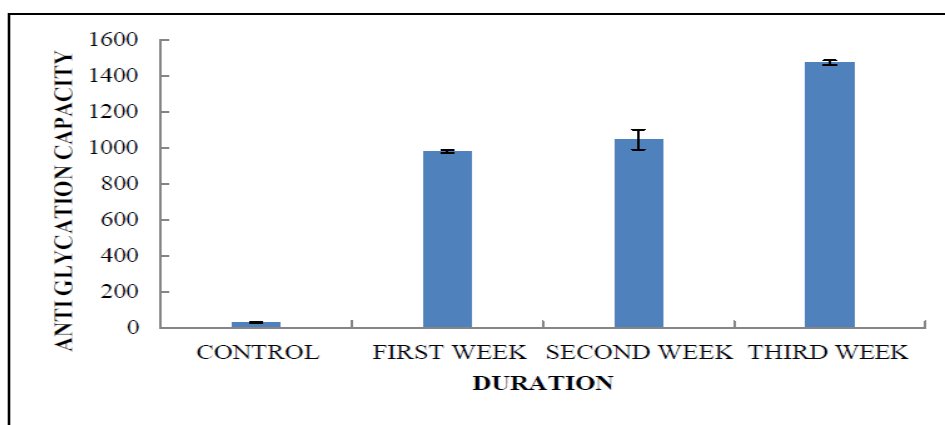


Fig 1: Antiglycation capacity of Garlic.

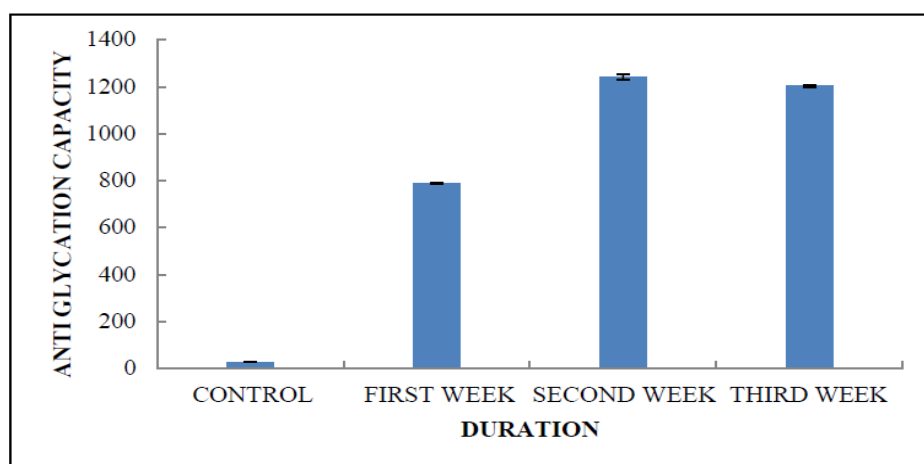


Fig 2: Antiglycation capacity of Cloves.

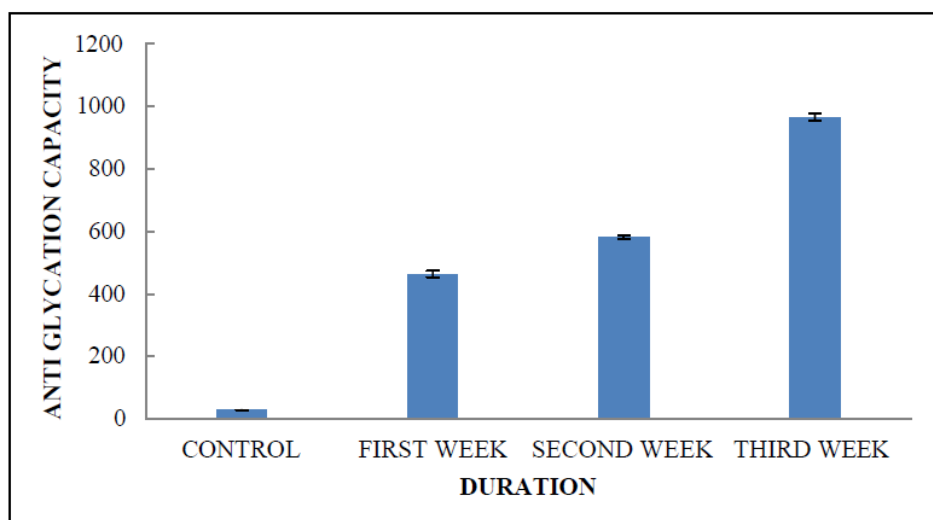


Fig 3: Antiglication capacity of Red chili.

CONCLUSION

It is concluded that these plants have antioxidant activity as well as advanced glycation end products (AGEs) inhibitory effects on phosphate-buffered fructose and BSA reaction. As a result, these plants could be offered as leading compounds for further study as a new products drug for diabetic complications.

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