# Contemplate Synthetic and Herbal origin provisions for non-enzymatic Glycation (NEGs) inhibition

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## Introduction

Diabetes in urban Indians is reaching an epidemic proportion and the prevalence of type-2 diabetes mellitus (DM) in Indians ranges from 2.7% in rural India to 14% in urban India. The prevalence of diabetes for all age-groups worldwide was 2.8% in 2000 and estimated to be 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030.[1] DM is higher, 26.9%, in the elderly (65 years and above). But it is also rapidly becoming a disease observed in younger patients with almost 2 million over the age of 20 being newly diagnosed with DM in 2010. More alarmingly, 35% of adults over the age of 20 and 50% of the elderly had pre-diabetes.[2] In healthy individuals, the blood glucose concentration is tightly regulated by insulin.[3] The high blood sugar concentration that is found during diabetes is related to either insufficient insulin production (i.e.,Type-1 diabetes) or resistance to insulin (i.e., Type-2 diabetes).[4,5] This increased blood glucose concentration has a number of effects in the body, which include an increased risk of heart disease,[6] stroke,[7] kidney disease,[8] blindness,[9] and amputations.[10] Many of these complications are due to protein glycation and the formation of advanced glycation end (AGE) products.[11] AGE-related complications in the body can be classified into two different areas. In one case, the interaction of AGEs with the receptor protein for AGEs (RAGE) is responsible for a wide range of inflammatory responses which eventually lead to tissue damage.[12,13] The second case involves direct modification of proteins by AGEs, leading to a loss of protein function[14] or tissue damage that results from protein cross-linking.[15] Different types of AGEs are known, depending on the compound they originate from. Six distinct classes of AGEs were recognised by Takeuchi *et al*, in (2004).[16]: deriving from glucose (AGE-1), from other carbohydrates, such as glyceraldehydes (AGE-2), and from $\alpha$-dicarbonyls, such as glycoaldehyde (AGE-3), methylglyoxal (AGE-4), glyoxal (AGE-5), 3-deoxyglucosone (AGE-6).

AGEs are implicated in many age related diseases such as type-2 diabetes mellitus, cardiovascular disease (the endothelial cell, collagen, fibrinogen are damaged), Alzheimer's diseases (amyloid protein are side product of the reaction progressing to AGEs), Cancer (acrylamide and other side product are related), peripheral neuropathy (the myelin is attached), and other sensory losses such as deafness (due to demyelination), and blindness (mostly due to micro-vascular damage in the retina). This range of diseases is the result of a very basic level at

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which glycation interferes with molecular and cellular functioning throughout the body.[17,18] An important part of tissue damage and cell death associated with chronic hyperglycaemia, and diabetes is mediated by free radicals. Extra cellular matrix (ECM) proteins such as collagen, elastin, actin, and myosin are the backbone for architectural and functional stability of tissues, cells and organs. Accumulation of AGEs, particularly in the ECM proteins, result in intra and inter molecular cross-linking leading to stiffening of these proteins and this is believed to play an important role in the aetiology of various AGEs related diseases.[19] Despite the availability of the current therapies for prevention of the protein glycation, oxygen stress related diseases are still a major threat to human health. Antioxidants effectively protect against glycation derived free radicals and may have therapeutic potential for the inhibition of radical induced processes. Moreover, plant samples with combined antioxidant and anti-glycation properties are highly desired because they can be more effective in treating various biological disorders. The current article reviews pharmaceutical and nutraceutical based molecules which have the potential to cause anti-glycating effect to NEGs and AGEs formation.

**Key words:** NEGs, AGEs, hyperglycaemia, glycation, Amadori products, Herbal drugs

## Inhibitors against AGE formation

Perhaps the most promising approach to AGE inhibition is the prevention of AGE formation. Besides the end-products, highly reactive intermediates responsible in their formation, and which are toxic to the cells such as glyoxal (GO), methylglyoxal (MGO), glycolaldehyde (GLA), and CML, N-carboxyethyllysine could also be targeted in designing inhibitors that specially react with each committed step as well as the intermediate products of important pathways.[20] Another factor to be considered in the search and development of AGE inhibitors is the fact that glycoxidised proteins generate reactive oxygen species (ROS), and induce oxidative stress through the reaction with RAGE. Also, ROS are generated by other reaction cascades of AGE formation such as MG, and Schiff base pathways leading to lipo-oxidation and oxidative damage.[21] Thus antioxidants also may be considered to have a role in the inhibition of non-enzymatic glycation.

## Non-enzymatic glycation Inhibitors and their classification

An efficient inhibitor of non-enzymatic glycation should inhibit glucose-derived AGE generation and cross-link formation.[22] A large number of synthetic and natural "AGE Inhibitors" have been reported. The mechanisms of non-enzymatic glycation or AGEs formation and cross-linking involve complex sequential and parallel reactions that are poorly understood.[23,24] A simple scheme that identifies common targets for AGEs inhibition, a guide and basis for rational design of new AGE inhibitors, and classification of existing AGE inhibitors.[23,24] AGE inhibitors can be classified in six categories or types.[25]

1. **Type-A:** sugar competitors are compounds that react with free amino groups of protein to modify them in order to prevent sugar attachment to protein (amino group capping agents).
2. **Type-B:** This class of inhibitors reacts with aldose and ketose sugars, inactivating them before they react with amino groups in the proteins. These compounds act on more than one step in the glycation cascade.[26]
3. **Type-C:** Antioxidants and metal chelators. This class of AGE inhibitors is likely to interfere with other types of reaction *in-vivo*. The C-1 type would be chelators such as DETAPAC, phytate, desferoxamine and penicillamine,[24] and the C-2 type inhibitors would be antioxidants, such as vitamin C or E.
4. **Type-D:** Inhibitors such as AG and L-arginine,16 trap reactive dicarbonyl intermediates (GO, MGO, glycoaldehyde and glucosones) to form substituted triazines. Some reactive dicarbonyls may be elevated in diabetics through metabolic imbalance distinct from non-enzymatic glycation.
5. **Type-E:** Amadori adducts inhibitors, it includes AG (Type E1), and also compounds that have potential for enzymatic de-glycation at the Amadori level.[27]
6. **Type-F:** This type includes cross-link breakers. These reduces AGE toxicity by breaking protein-AGE cross-linking.

There are also numbers of novel AGE inhibitors such as zinc and nano-particles, whose inhibition mechanisms are still under investigation.

## Synthetic sources of glycation inhibitor

There are several commercially available (synthetic) inhibitors of cross-linking. Examples of these include carnosine, aminoguanidine, metformin, and pyridoxamine. Some of these (like acarbose and metformin) are already in use as anti-diabetic drugs. Other not yet widely available inhibitors are Tenilsetam, OPB9195, and several others still in development.[28] The Alteon Corporation alone has identified over 850 separate cross-link inhibitors.[29]

However, the first compound which has been extensively studied *in-vitro* and *in-vivo* for inhibition of AGEs formation is aminoguanidine, (Pimagidine). Aminoguanidine works by forming guanidine-dicarbonyl adducts, thereby reducing the numbers of free carbonyl groups. In particular, it is active against certain aldehydes which contribute to cross-linking, (e.g. alpha-oxoaldehyde, and malondialdehyde). Aminoguanidine is active mainly during the early stages of glycosylation. It is an effective inhibitor of cross-linking initiated by glucose

molecules, but not as effective in situations involving ribose-related cross-linking. Among the side effects reported in patients treated with AG was pernicious-like anaemia,[30] and also for the development of anti-nuclear antibodies (ANA) in high-dose AG therapy. Also, higher rates of pancreatic and renal-neoplastic tumours were reported in diabetic rats treated with AG. Unfortunately, the clinical phases in the AG treatment of patients with type-1 diabetes were ended due to serious complication in the patients.[31]

The *in-vitro* effects of three existing drugs, metformin, pioglitazone and pentoxyfylline have been evaluated by Rahbar and Figarola[32] on AGE formation and they demonstrated that all the three drugs are potent inhibitors of glycation.[33,34] Gramicidin-S, a cyclic decapeptide, was used as a model for the study of protein glycation and its reversal in the presence of nucleophilic amines. Gramicidin-S [cyclo(Leu-d-Phe-Pro-Val-Orn)2] is a structurally well-defined peptide which is constrained to adopt an anti-parallel sheet structure with cyclisation facilitated by two d-Phe-Pro a-turns. In this conformation, the alkylamino side chains on the two ornithine residues lie on the same phase of the a-sheet in close proximity.[35,36] They show, using electrospray ionisation mass spectrometry (ESI-MS), multiple glycation and formation of advanced glycation product in gramicidin S and also rapid reversal of Shiff base using hydrazines such as hydroxylamine, aminoguanidine and isonicotinic acid hydrazide.[34] Metformin (brand names Glucophage, Metforal) is a standard anti-diabetic drug (dimethyl-biguanide) used worldwide both against insulin-dependent and against non-insulin-dependent diabetes.[36] In a clinical trial examining 57 people with type 2 diabetes, treatment with metformin was shown to reduce the concentration of methylglyoxal in a dose dependent manner.[37] A derivative of vitamins-B6, pyrodoxamine has similar mechanism of action i.e. scavenging of dicarbonyls, thus preventing their conversion to AGEs. It prevented renal dysfunction in diabetic rats and was highly effective in reducing retinal AGE accumulation.[38,39] It also prevents the up-regulation of glomerular basement membrane-associated genes and diabetes-associated genes and diabetes-associated capillary dropout.[40] Pyridoxamine and thymine inhibit the post Amadori steps of Maillard reaction by sequencing catalytic metal ions and blocking oxidative degradation of Amadori intermediates.[41] Pyridoxamine also inhibits chemical modification of proteins by scavenging carbonyl intermediates of carbohydrates and lipid degradation.[42] Other compounds having similar activity to AG are D-penicillamine[43] and desferoxamine, perhaps due to metal chelating properties.[44] Anti-inflammatory compounds such as acetylsalicylic acid, Ibuprofen, and indomethacin were reported to be inhibitors of glycation, perhaps by preventing the oxidative stress associated with formation of AGE.[45] Zhang *et al*., performed a comparative study to see the protective effect of aspirin. Aspirin caused a reduction in glycation probably by acetylation of amino acids *in-vitro*, and in animal models.[46] So it may protect against glycation.[47] According to the Duraisamy *et al*., study, compounds with combined anti-glycation and anti-oxidant properties such as aminosalicylic acid are more effective than aminoguanidine in protecting against the harmful effect of high glucose and AGE *in-vitro*.[48] In the comparison study performed by Zhang *et al*.,(2010) Diclofenac (Voltaren), a non-steroidal anti-inflammatory drug was studied *in-vitro*. It was found to be an inhibitor of AGE.

Ramipril attenuated the renal AGE accumulation found in streptozotocin (STZ) induced diabetic rats, identifying a relationship between the renin-angiotensin system and the accumulation of AGEs in experimental nephropathy.[49] Furthermore, treatment with ramipril has been shown to lower serum AGEs.[50] Another compound that prevents the formation of AGEs is thiamine (and its lipid soluble derivatives, benfotiamine), which has shown great promise in animal models[51] by increasing transketolase activity (the rate-limiting step of non-oxidative branch of the pentose phosphate pathway). Thiamine reduces the availability of glyceraldehyde-3-phosphate and fructose-6-phosphate, and thereby reduces dicarbonyl formation.[52] Benfotiamine was found to be a potent inhibitor of glycation in STZ induced diabetic rats and experimental diabetic nephropathy.[49] In rats long term administration of benfotiamine resulted in decreased vascular abnormalities in the retina and attenuated the activation of NF-κB.[51] Di-peptide carnosine (β-alanyl-L-histidine) is a naturally-occurring agent found in muscle and nervous tissue. Carnosine has been hailed as one of the most promising cross-link inhibitors. Another important carnosine activity is 'carnosinylation', which is a process whereby carnosine attaches to the proteins bearing a carbonyl group, thus blocking the carbonyl from attaching to another protein. In other words, Carnosine has a direct antioxidant action, and it also has a sparing effect on other antioxidants such as glutathione. It is a strong chelator of copper thereby reducing the copper-mediated damage during AGE activity. At the clinical level, carnosine reduced urinary products of free radical and glycosylation metabolism in humans. One of the most important developments regarding carnosine is its ability to prevent and cure age-related cataract, and possibly glaucoma and other age-related eye conditions.[53] Tenilsetam (3-2-thienyl-2-piperazinone) manufactured by Cassella, a subsidiary of Aventis, has traditionally been used as a brain stimulant (nootropic). Tenilsetam has antioxidant activities and copper chelating properties. More recent experiments show that Tenilsetam reduces AGEs in diabetic rats, reduces amyloid aggregates (amyloid is the result of brain protein cross-linking), prevents oxidation injury to the brain and

has an overall anti-dementia effect.[54]

In last few years, compounds mostly, derivatives of aryl, ureido, and aryl carboxaminido phenoxy isobutyric acid, were screened and evaluated using several well established *in-vitro* assay methods.[55]

## Cross-link Breakers

Cells and tissues constantly exposed to AGEs tend to lose their function due to protein cross-linking. A few AGE breakers have been discovered to combat deleterious protein cross-links. The most important cross-link breaker is the drug ALT-711, an orally active compound. This is a thiazolium product (dimethyl-3-phenacyl-thiazolium chloride) manufactured by the Alteon Corporation in the US. A related compound is PTB (dimethyl-Phenacyl-Thiazolium Bromide), which has actions similar to the chloride variety. ALT-711 is not an enzyme as such, but it has enzymatic properties. It has been shown to actually break the covalent bonds between cross-linked proteins and free the proteins which are then able to function again normally.[56]

Particularly, ALT-711 breaks the bonds between -O=C-C=O-, (the first -O=C group belonging to one protein and the second C=O- belonging to another). When the bond between C-C is broken, the first protein has a -COOH group and the second protein has a -CHO group. Although, in theory, the bonds may then re-form, because the carbonyl group is still active on the freed protein. In other words, if the proteins are cross-linked again, ALT-711 will divide them once more, and if they are then re-bound, it will keep on separating them. For this reason, it may be necessary to use a combination of the cross-link inhibitor carnosine together with ALT-711 for full protection against cross-linking. In that situation, when the C-C bond is broken, carnosine will immediately bind to the carbonyl group (i.e. it will 'carnosinylate' the protein) and therefore cross-linking of that particular protein will not take place for the second time. ALT-711 can reverse aortic stiffening in rodents, canines and primates. Other experiments support its effectiveness against hypertension, cardiovascular stiffness and heart failure.[56] It has also been studied in a number of human clinical trials. It was found to be effective in reversing some of the complications of diabetes, improving myocardial and arterial stiffness, heart failure, and reducing blood pressure.[57]

Preliminary reports are optimistic, showing that ALT-711 is effective at reducing clinical symptoms. Statistically significant reduction of blood pressure and an increase in large artery compliance was achieved after an eight week treatment period. The drug was well tolerated and few side effects were reported. Other trials are in progress aiming to study ALT-711 in relation to diabetes and skin ageing. Far from being unique; ALT-711 is in a group of 375 other cross-link breakers developed by Alteon.[29]

## AGEs inhibitors of herbal origin

Plants have been used from ancient times to attempt cures for diseases and to relieve physical suffering. Ancient people had acquired some knowledge of medicinal plants.[58] Medicinal plants-phytochemicals have the advantage of having little or no side effects. With the increasing popularity of herbal medicine, physician should have at least some basic knowledge about traditional medicine. Furthermore, some of the traditional plant based remedies or at least components derived from medicinal plants or herbs, may one day become part of mainstream treatments.[59]

There are reports of some natural substances or phy-

**Table-1: Medicinal plants used in non-enzymatic glycation inhibition activity**

| Medicinal plants | Mode of action for AGEs inhibition | Class of inhibitors | References |
|---|---|---|---|
| *Withania Somnifea* | Scavenge free radicals and inhibit lipid peroxidation | C1 | [67] |
| *Allium sativaa,* | Antioxidant activity | C2 | [68] |
| *Plantago asiatica* | Antioxidant activity | C2 | [69] |
| *Pueraria lobata* | ----- | ---- | [70] |
| *Phyllanthus emblica* | Antioxidant activity | C2 | [71] |
| *Kaempferia . parviflora* | Antioxidant /metal chelators | C1 and C2 | [66] |
| *Globba. wintii* | Antioxidant /metal chelators | C1 and C2 | [66]) |
| *Hibiscus cannabinus* | Antioxidant activity | C2 | [72] |
| *Eulophia nuda* | Inhibit Maillard products | E | [73] |

tochemicals isolated from plants with non-enzymatic glycation or AGEs inhibitory effects. The *Curcuma longa* rhizomes have been reported to possess anti-diabetic properties as its alcoholic extracts possess active constituents showing blood glucose lowering activity in alloxan induced diabetic rats.[60] The purified curcumin was more effective than turmeric and it has antioxidant and anti-inflammatory properties.[61] The screening of plant extracts with anti-oxidative properties, such as, curcumin, rutin, garcino yield flavonoid-rich extracts, which have been shown to prevent AGEs formation in *in-vitro* and *in-vivo* experiments.[62,63] Arbutin (hydroquinone-β-D-glucopyranoside) is a naturally occurring compound found in various plant species of diverse family such as Ericaceae (Arctostphylos spp.), Betulaceae (Betula alba) and Rosaceae (Pyrus communis L.).[64] Arbutin, shows *in-vitro* antiglycation activity.[65] Extracts of 24 herbs and spices were tested for the ability to inhibit glycation of albumin. The most potent inhibitors included extracts of cloves, ground Jamaican allspice, and cinnamon, sage, marjoram, tarragon, and rosemary. The concentration of phenolics that inhibited glycation by 50% was typically 4–12 μg/ml. Relative to total phenolic concentration extracts of powdered ginger and bay leaves were less effective than expected, and black pepper was more effective.[66]

## Conclusion

The standard treatment of NEGs and AGEs continues to be eminently therapeutic. For patients with diabetic complication approaches provide an alternatively to synthetic and herbal drugs with comparable results in various *in-vitro* and *in-vivo* models. Synthetic drug treatments lead to unwanted side effects, and hence the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes. Hence, plants have been suggested as a rich, as yet unexplored source of potentially useful anti-glycating drugs. However, only a few have been subjected to detailed scientific investigation due to a lack of mechanism-based *in-vitro* assays. Nutraceuticals have been shown to improve the outcome in hyperglycaemia complication with NEGs and AGEs formation. There is growing evidence that show improvement in NEGs and AGEs related complications. In the future, targeting the NEGs and AGEs with phytochemicals which have anti-glycating activity by applying QASAR, molecular docking and other bioinformatic tools for pharmacological evaluation. The Bio-pharmaceutics may play major role in the treatment of these conditions in near future.

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## ERRATA

1. Vol 67, No.8, Aug 2014 of The Indian Practitioner

   In Case Report titled 'Secondary MDR Tuberculosis of the Breast' page 509 the name of only one author, Nathe A.S. was mentioned and the names of the co-authors: Reena Set, Nishat Khan and Jayanthi Shastri, Department of Microbiology, T. N. Medical College were inadvertently left out. The error is regretted.

2. Vol 67, No.9, Sept 2014 of The Indian Practitioner

   In Case Report titled 'Streptomycin monoresistant extrapulmonary tuberculosis in an immunocompetent healthcare worker' page 566, the name of the co-author should read as 'Set R' and not 'Seth R', as published. The error is regretted.