

# Inhibition of Advanced Glycation End-Product Formation by Lutein from *Tagetes erecta*

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## ABSTRACT

**Background:** Advanced glycation end products (AGEs) are formed by non-enzymatic glycation of proteins that enhance vascular permeability in both micro and macro vascular structures by binding to specific macrophage receptors. AGEs affect nearly every type of cells and molecule in the body and play causative role in the vascular complication in diabetes mellitus. The present study was carried out to evaluate the effect of lutein extract on Advanced glycation end product. **Materials and Method:** AGE reaction solution was constituted with 10mg/ml bovine serum albumin in 50mM sodium phosphate buffer (pH 7.4) and 0.02% sodium benzoate into 0.2M fructose and 0.2M of glucose. 2.5 ml of the reaction mixture was treated with lutein (10, 20, 40, 60, 80, 100, 120, 140  $\mu$ g/ml in methanol). Amino guanidine was used as the positive control. After incubating at 37°C for 7 days, the fluorescence intensity of the reaction was determined at excitation and emission wavelength of 350 nm and 450 nm, respectively, using a multi-mode reader. The percentage activity was calculated with respect to solvent control. **Result:** 7 days of exposure to lutein showed a maximum inhibition of 89.27 $\pm$ 0.24 % at 140 $\mu$ g/ml and Amino guanidine exhibited 90% of inhibition. The IC<sub>50</sub> of Lutein for AGE inhibition was found to be 77.78 $\mu$ g/ml and for AG, 72.66 $\mu$ g/ml. **Conclusion:** The non-enzymatic adduct formation between the keto group of sugar and amino group of proteins is one of the molecular basis of diabetic complications in hyperglycaemic state. Inhibition of this process will be useful in the management of diabetic complications. Lutein showed dose dependent inhibitory effect on the protein glycation. Hence, it may be used for the management of diabetic complication.

**Key words:** Lutein, Amino-guanidine, Advance glycation end product, Diabetes mellitus.

## INTRODUCTION

Advanced glycation end-products are a complex and heterogeneous group of compounds that have been implicated in diabetes related complications. Reducing sugars such as glucose, react non-enzymatically with amino groups in proteins, lipids and nucleic acids through a series of reactions forming Schiff bases and Amadori products to produce AGE. Hyperglycaemia has been implicated in the accelerated vascular damage associated with diabetes, which eventually manifests micro vascular complications such as retinopathy, neuropathy, nephropathy and macro vascular disease.<sup>1</sup> In early stage unstable Schiff base forms and, through acid base catalysis this compound undergoes further rearrangement to a more stable Amadori product and via dehydration, oxidation reaction and different chemical reactions degrades to more reactive carbonyl compounds and they act as propagators of the reaction again reacting with more free amino groups of biomolecules. In the late stage Advanced Glycation End Products (AGEs) are formed. Various studies have shown that diabetes mellitus is associated with an increased production of free radicals leading to oxidative stress. Thus, disturbed balance between radical formation and radical neutralisation leads to oxidative damage of cell components such as proteins, lipids and

nucleic acids. Oxidation plays an important role in the formation of Advanced Glycation End product and the plant derived agents with the antiglycation and antioxidant activities are highly important in preventing diabetic complications.<sup>2</sup> Diabetic complications are considered to be multifactorial in origin with increasing evidence that one of the major pathways involved in the development and progression of both micro vascular and macro vascular disease as a result of chronic hyperglycaemia is the biochemical process of advanced glycation. The effects of AGEs may be classified as receptor-independent or -dependent and can act intracellularly or circulate and act on cell surface receptors such as the receptor for AGEs (RAGEs). Advanced glycation occurs over a prolonged period, affecting long-lived proteins. AGEs are a complex group of compounds formed via a non-enzymatic reaction between reducing sugars and amine residues on proteins, lipids, or nucleic acids.<sup>3</sup> AGEs are also known to be involved in the development of Alzheimer's and other neurological diseases, as well as cardiovascular diseases, including atherosclerosis, endothelial dysfunction, and vaso-relaxation. However, although numerous AGE inhibitors, including the

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well-known amino-guanidine, ameliorated diabetic complications in animal model, they also caused severe side effects including gastrointestinal disturbance, anaemia and flu-like symptoms. Natural products represent an important option for the discovery and development of new anti-AGE pharmaceuticals; these include metabolites with antioxidant activity since oxidative stress is involved and accelerates the formation of AGE. The complexity in the secondary metabolism of plants which results in a great chemical diversity of natural products, are recognised as a vital source of bioactive metabolites. In addition, since many plants are traditionally used to treat diseases such as diabetes and other disorders associated with AGE formation, these particular species constitute a potentially important source for the search of novel secondary metabolites with anti-AGE activity.<sup>4</sup> Both synthetic compounds and natural products have been evaluated as inhibitors against the formation of advanced glycation end products (AGEs). The synthetic AGEs inhibitors so far discovered are divided into three classes: (a) carbonyl trapping agents which attenuate carbonyl stress; (b) metal ion chelators, which suppress glycoxidations; and (c) cross-link breakers that reverse AGE cross-links. Though these drugs are known for their inhibitory capacities against the formation of AGEs, many synthetic inhibitors of AGEs formation were withdrawn from clinical trials due to relatively low efficacies, poor pharmacokinetics and unsatisfactory safety. For example, amino-guanidine (AG), a nucleophilic hydrazine compound which prevents the formation of AGEs was withdrawn from the crucial phase III of clinical trials because of safety concerns and apparent lack of efficacy. On the other hand, natural products have been proven relatively safe for human consumption and many plant extracts have been tested for their ability to prevent AGEs formation. Results showed that lutein and some unsaturated fatty acids effectively inhibited the formation of both total AGEs and specific AGEs *in vitro* in a dose-dependent manner.<sup>5</sup> Though Amino guanidine hydrochloride inhibits the formation of advanced glycation end products (AGEs) *in vitro* and *in vivo*,<sup>6</sup> Numerous epidemiological studies support a strong inverse relationship between consumption of carotenoid-rich fruits and vegetables and the incidence of some degenerative diseases. One proposed mechanism of protection by carotenoids centres on their putative antioxidant activity, although direct evidence in support of this contention is limited at the cellular level. The antioxidant potential of  $\beta$ -carotene (BC) and lutein (LUT), carotenoids with or without provitamin A activity, respectively, was evaluated using the human liver cell line HepG2.<sup>7</sup> The present study investigated the potential inhibitory activity of lutein powder from *Tagetes erecta* on protein glycation.

## MATERIALS AND METHOD

### Drugs and chemicals

Bovine serum albumin, sodium phosphate buffer, sodium benzoate, fructose, glucose, amino guanidine. All the drugs and chemicals used were of analytical grade.

### The plant extracts

Lutein was obtained from Synthite Industries Limited, Kerala as gratis. The extract was evaluated for its activity on AGEs formation. AGE reaction solution was constituted as follows; 10mg/ml bovine serum albumin in 50mM sodium phosphate buffer (pH 7.4) and 0.02% sodium benzoate into 0.2M fructose and 0.2M of glucose. The reaction mixture (2.75ml) was treated with lutein (10, 20, 40, 60, 80, 100, 120, 140  $\mu$ g/ml in methanol). Amino guanidine was as positive control. After incubating at 37 degree Celsius for 7 days, the fluorescence intensity of the reaction was determined at excitation and emission wavelength of 350 nm and 450 nm, respectively, using a multi-mode reader (PerkinElmer Enspire, USA). The percentage activity was calculated with respect to solvent control.

## RESULTS

In the present study, the effect of lutein against protein glycation was evaluated and its potency was compared with standard anti-glycation agent, amino-guanidine (AG). 7 days of exposure to lutein showed a maximum inhibition of  $89.27 \pm 0.24\%$  at 140  $\mu$ g/ml and AG exhibited 90% of inhibition (Table 1 and Figure 1). The  $IC_{50}$  of lutein for AGE inhibition was found to be 77.78  $\mu$ g/ml and for AG, 72.66  $\mu$ g/ml.

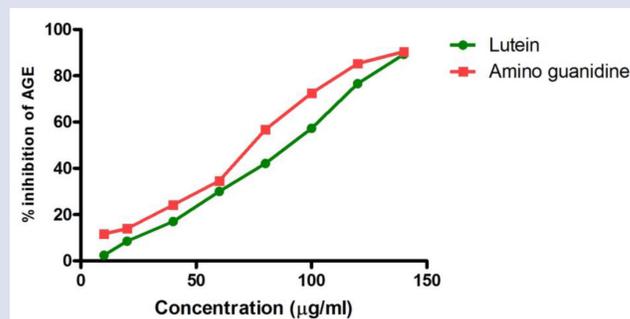
## DISCUSSION

In the above study, lutein extracted from the plant marigold (*Tagetes erecta*) was compared with amino guanidine for its inhibitory activity on the advanced glycation end products. The study focused on the inhibition of protein glycation product. Glycation is a post-translational modification resulting from the interaction of protein amino and guanidine groups with carbonyl compounds. Protein glycation, is a non-enzymatic post-translational modification formed by interaction of lysyl and arginyl residues with carbonyl compounds such as carbohydrates and  $\alpha$ -dicarbonyls.<sup>8</sup>

Lutein from *Tagetes erecta* is a purified extract obtained from marigold, which is extracted from the petals of marigold flowers with organic solvents. Lutein is an oxycarotenoid, or xanthophyll, also known as vegetable lutein; vegetable luteol; Bo-Xan; all-trans-lutein; 4', 5'-didehydro-5', 6'-dihydro-beta, beta-carotene-3, 3'-diol, containing 2 cyclic end groups, one beta- and one alpha-ionone ring and the basic C40 isoprenoid structure common to all carotenoids. In many studies lutein showed marked increase in tumour latency, suppressed mammary tumour growth and enhanced lymphocyte proliferation.<sup>9</sup> In many research, lutein showed a marked inhibition of the complication related to dia-

**Table 1: Effect of Lutein on Protein glycation and inhibitory potential**  
Values are expressed as Mean  $\pm$  SEM (n = 3).

concentration of lutein [ $\mu$ g/ml]	% Inhibition of AGEs
10	2.47 $\pm$ 0.33
20	8.53 $\pm$ 0.35
40	17.07 $\pm$ 0.19
60	30.06 $\pm$ 0.97
80	42.11 $\pm$ 0.73
100	57.30 $\pm$ 0.49
120	76.63 $\pm$ 0.44
140	89.27 $\pm$ 0.24



**Figure 1:** Graph showing inhibitory effect of Lutein on Advanced Glycation End product.

abetic patients like prevention the effect of high glucose levels on immune system cells *in vivo* and *in vitro*.<sup>10</sup> It even helps in eye diseases, age related muscle degeneration retinitis pigmentosa. Here, lutein was compared with the standard drug amino-guanidine for its inhibitory action on protein glycation.

Similar studies are done on some other plants for their inhibitory action on glycation and it includes wheat bran, guava-leaf etc. Protein glycation is believed to play an important role in the development of long-term disorders associated with diabetes. Water-soluble feruloyl oligosaccharides from wheat bran, the ferulic acid esters of oligosaccharides, have been reported as natural antioxidants.<sup>11</sup> The potency of Polyphenon 60 which is a commercial polyphenol product extracted from green tea was compared with the standard antiglycation agent, amino guanidine. The results showed that the inhibitory effects of guava leaf extracts on the formation of  $\alpha$ -dicarbonyl compounds were over 95% at 50  $\mu\text{g/ml}$ .<sup>12</sup> Leaf extract of the plant *Caralluma fimbriata* had proven Anti-hyperglycaemic activities in *in-vitro* studies.<sup>13</sup> Anti-diabetic plant *Butea monosperma* had shown a marked inhibition of aldose reductase in diabetic rats.<sup>14</sup> Plants are thus immensely explored for their therapeutic effectiveness.

Natural products represent an important option for the discovery and development of new anti-AGE pharmaceuticals; these can include metabolites with antioxidant activity since oxidative stress is involved and accelerates the formation of AGEs. Plants are proved to have anti-diabetic, anticancer and anti-anxiety properties.<sup>15-18</sup> Diet is also an important contributor to the circulating AGE pool in health and in the disease state.<sup>19</sup> The antiglycation activities of both plant materials and naturally occurring phenolic compounds with antioxidant properties have been investigated.<sup>20</sup>

## CONCLUSION

The non-enzymatic adduct, formation between the keto group of sugar and amino group of proteins is one of the molecular basis of diabetic complications in hyperglycaemic state. Inhibition of this process will be useful in the management of diabetic complications. Lutein from *Tagetes erecta* showed dose dependent inhibitory effect on the protein glycation. As the plant demonstrated a significant ability to inhibit the formation of advanced glycation end-product *in vitro*, it may be used for management of diabetic complication after making a proper formulation and further validation.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

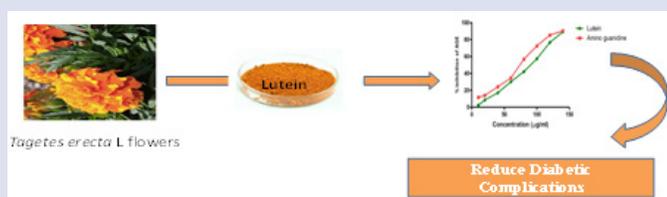
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## GRAPHICAL ABSTRACT



## SUMMARY

- Lutein from *Tagetes erecta* L has significant inhibitory effect on advanced glycation end product and will be beneficial for diabetic patients to reduce the later stage complications. It can be used in the formulation of diabetic drugs.

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