



Manuscript 1048

Watermelon seed extract inhibits protein aggregation and formation of advanced glycation end-products

Faiyaz Ahmed

Satish A

Follow this and additional works at: <https://rescon.jssuni.edu.in/ijhas>

ORIGINAL STUDY

Watermelon Seed Extract Inhibits Protein Aggregation and Formation of Advanced Glycation End-products

Faiyaz Ahmed ^a, Satish Anandan ^{b,*}

^a Department of Clinical Nutrition, College of Applied Health Sciences in Ar Rass, Qassim University, Al Qassim Region, 51921, Saudi Arabia

^b Department of Clinical Nutrition and Dietetics, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India

Abstract

Background: Glycation of proteins is a key element in the onset of diabetes consequences. The potential of medicinal plants to inhibit the progression of glycation complications provides a significant opportunity as an alternative therapy.

Methods: The effect of watermelon seed extract (WMSE) on antioxidant capacity was assessed based on radical scavenging activity and metal chelation activity. Further the inhibition of Advanced glycation end-products (AGEs) formation was assessed by using different sources of amine (haemoglobin, bovine serum albumin, G.K peptide) and sugar sources (δ -gluconolactone, methylglyoxal, ribose). Aminoguanidine (AG) used as standard drug.

Results: Due to the presence of various phytochemicals that could disrupt the chain of events and prevent the development of AGEs, WMSE demonstrated its potential inhibitory effects at every step of protein glycation. A key part of this response is the scavenging of free radicals produced by glycation.

Conclusion: The WMSE may aid to give a protective effect against protein degradation caused by hyperglycemia, which may be helpful in the treatment of problems associated with diabetes.

Keywords: *Citrullus lanatus*, Watermelon seed, Glycation, Antioxidant

1. Introduction

A fundamental flaw in glucose metabolism in biological systems is frequently linked to protein glycation and post-translational mutation. The development of pathological disorders like atherosclerosis, diabetes complications, skin disorders, rheumatism, hypertension, and neurodegenerative diseases is thought to be facilitated by complicated heterogeneous sugar-derived protein changes known as AGEs [1]. Undoubtedly, there is a need to increase our understanding of anti-glycation drugs and the therapeutic approaches they can provide for the prevention and management of serious social and economic health problems. Although many different compounds have been

considered, there aren't many approved and registered anti-glycation medications, and there isn't much information from clinical trials. In addition to the search for novel synthetic AGEs inhibitors, scientists are increasingly focusing their efforts on natural anti-glycation compounds [2].

The tropical fruit known as the water melon (*Citrullus lanatus*) is a member of the cucumber family (Cucurbitacea). It is a major horticultural crop that is primarily grown in warm climates because of the pulp's sweet and juicy flavour [3]. However, the seeds and rinds are not consumed with the pulp; they are often thrown away or used for animal feed. In the juice processing industry, fruits that are edible are processed into a wide range of products such as puree and canned slices, although seeds are frequently discarded as waste. This waste causes

Received 22 February 2023; revised 24 June 2023; accepted 24 July 2023.
Available online 28 September 2023

* Corresponding author at: Department of Clinical Nutrition and Dietetics, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India.
E-mail address: satishanandan84@gmail.com (S. Anandan).

<https://doi.org/10.55691/2278-344X.1048>

2278-344X/© 2023 JSS Academy of Higher Education and Research. This is an open access article under the CC-BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

ecological issues due to the spread of insects and rodents; thus, research exploring potential uses for this material is essential [4]. Recently, there has been a greater emphasis placed on the possible use of agricultural by-products in the creation of new functional ingredients for food fortification that will provide an alternative for industries and promote environmental sustainability [5]. Frequently, waste products from agriculture are sources of phytochemicals with functional properties, like saponins, phenolics, and alkaloids which can scavenge free radicals in biological systems [6]. With consideration of the nutrients and phytochemical content, the use of agricultural waste to reduce environmental pollution has increased [7]. There is a lack of knowledge on the phytochemicals and radical scavenging abilities of watermelon seeds, despite the abundance of research on watermelon seed oil and its proximate contents. However, there is currently no research on the anti-glycation effectiveness of water melon seed extract against protein-induced glycation. So, using appropriate *in-vitro* techniques, we attempted to investigate the antioxidant activity, metal ion chelating activity, and anti-glycation activity from an aqueous extract of watermelon seed.

2. Materials and methods

2.1. Materials

Shelled water melon (*Citrullus lanatus*) seeds were purchased at a local vendor in Kolar, India.

2.2. Chemicals

Acetylglycyl-lysine methyl ester (G.K.) peptide, aminoguanidine hydrochloride (AG), Thioflavin-T dye (Th-t), Bovine serum albumin (BSA), methylglyoxal (MGO-40% in H₂O), and δ -Gluconolactone (δ -glu) and other chemicals employed in the investigation were of analytical grade and were purchased from SRL (India).

2.3. Preparation of watermelon seed extract (WMSE)

The watermelon seeds were washed and dried over night in an oven at 37 °C, Shelled seeds were ground into flour using an electronic mill, sieved through a 60 mesh sieve, and kept at 4 °C until use. The powdered samples (50 g) will be extracted with distilled water (1:10 w/v) in a mechanical shaker for 24 h at room temperature. Further the extracts were filtered, freeze-dried, and kept at 4 °C until use.

2.4. Antioxidant activity

2.4.1. Radical scavenging assay

The radical scavenging activity (RSA) method was used to assess WMSE extracts' capacity to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals [8]. Briefly, 3 ml of extract (containing 50–300 μ g/ml) in methanol were combined with 1 ml of 0.1 mM DPPH solution. After a strong vortex mixing, the mixture was left at room temperature and in the dark for 30 min. The absorbance was measured at 517 nm, and the activity was represented as a % RSA in comparison to control using equation.

$$\% \text{ Inhibition} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100 \quad (1)$$

Where A_{sample} represents the absorbance when a WMSE is present and A_{control} represents the absorbance of the control reaction (without a WMSE).

By substituting 50 for y in the equation of linear regression created by the association between x = level and y = % inhibition, the value IC₅₀ can be calculated.

2.5. Determination of metal chelating activity

Measurements of metal chelating activity were performed as described previously [8]. Briefly, 0.2 mL of WMSE of various concentrations (50–300 μ g/ml) were added along with 0.25 mM ferrozine and 0.1 mM FeSO₄. After 10 min of incubation at room temperature, the mixture's absorbance was measured at 562 nm. Using the equation above, the IC₅₀ was computed as mentioned above.

2.6. Antiglycation activity

2.6.1. Early stage of protein glycation

Hemoglobin- δ -gluconolactone (HDG) assay - The HDG assay was carried out in accordance with our previously described protocol [9]. Briefly, about 800 μ L of fresh human blood samples were mixed with 0.1 M PBS (0.16 mL, pH 7.4) containing δ -glu (50 mM) and WMSE (300 μ g/mL) for a final volume of 1 ml was mixed thoroughly and subjected for incubation for 16 h at 37 °C. The amount of glycated hemoglobin in the controls and samples containing WMSE was determined by ion-exchange HPLC system as described in kit. Briefly, after preparing the hemolysate, where the labile fraction is eliminated, hemoglobins are retained by a cationic exchange resin. Hemoglobin A1c (HbA1c) is specifically eluted after washing away the hemoglobin A1a + b fraction1 (HbA1a + b), and is

quantified by direct photometric reading at 415 nm. The estimation of the relative concentration of HbA1c is made by the measure of total hemoglobin concentration by direct photometric reading at 415 nm.

2.7. Intermediate stage of protein glycation

2.7.1. BSA-methylglyoxal (MGO) assay

The inhibition potential of WMSE against protein glycation was determined using a previously established protocol [10]. Briefly, BSA (10 mg/ml) was incubated with 10 mM MGO in the absence or presence of 10 mM AG and 300 µg/ml WMSE under sterile conditions in 100 mM phosphate buffer (pH 7.4) at 37 °C for 24 hrs. In comparison to an unincubated blank containing the protein, MGO, and inhibitors, the fluorescence of the samples was evaluated at the excitation and emission maxima of 330 and 410 nm, respectively. Further, the formation of the amyloid fibril assay was also performed as our previously published procedure. Briefly, the freshly prepared ThT dye solution (1 mM) in 20 mM phosphate buffer (pH 7.4) was immediately added to the glycated samples and blank sets and incubated for 30 min at RT. The dyed samples (10 µL) were placed on glass slides and images were taken using a fluorescence microscope.

2.8. Last stage of protein glycation

2.8.1. N-acetylglycyl-lysine methyl ester (G.K.) peptide-ribose assay

The last stage of the glycation assay was carried out according to the previously described protocol [9]. This test will be used to evaluate the ability of WMSE (300 µg/ml) to inhibit the cross-linking of G.K. peptide (80 mg/mL) in the presence of ribose (0.1 M). Samples will be examined for particular fluorescence (excitation, 340 nm; emission, 420 nm) at the end of the incubation.

3. Results

3.1. Antioxidant activity

The effects of WMSE on various antioxidant properties are shown in Table 1. DPPH and metal ion chelation activity of WMSE showed significant radical scavenging properties and chelating Fe²⁺ ions in a dose-dependent manner (50–300 µg/ml). The IC₅₀ values for DPPH of WMSE and standard Butylated hydroxytoluene (BHT) were found to be 246.36 µg/ml and 55.63 µg/ml, respectively and IC₅₀ values for metal ion chelating of WMSE and standard

Table 1. DPPH and Metal chelating and IC₅₀ values of the WMSE.

SL.No.	DPPH IC ₅₀ Value		Metal chelating IC ₅₀ Value (µg)	
	BHT	WMSE	EDTA	WMSE
1	55.63	246.36	32.02	260.58

Ethylenediaminetetraacetic acid (EDTA) were found to be 260.58 µg/ml and 32.023 µg/ml, respectively.

3.2. Antiglycation activity

3.2.1. Haemoglobin-δ-gluconolactone (HDG) assay

The early stage of glycation, or the generation of amadori products, was assessed using ion-exchange HPLC (BIOSYSTEMS). Fig. 1a depicts the absence or presence of WMSE (300 µg/ml) in the HDG system. After incubation, the content of glycated haemoglobin in the HDG reaction mixture was significantly higher than in the consisting only haemoglobin (fresh blood). However, in the presence of WMSE in the HDG reaction mixture, the level of glycated haemoglobin gradually decreased.

3.2.2. BSA-methylglyoxal (BSA-MGO) assay

From fluorescence spectroscopic, It was observed BSA incubated with MGO increased the fluorescence intensity due to AGEs formation. The presence of WMSE decreased the fluorescence intensity indicating inhibition of AGEs formation. As can be seen from the low fluorescence intensity in Fig. 1b, in presence of WMSE reduced MGO-mediated albumin glycation. Further we used fluorescent microscopic imaging to understand more about how WMSE prevents albumin from aggregating using the ThT dye. This dye interacts to amyloid aggregates, which fluorescence in a blue-green colour. In the present investigation, control BSA (Fig. 2a) exhibited lower bright fluorescent images than BSA incubated with MGO (Fig. 2b). However, the protein's fluorescence was less intense when WMSE 300 µg/ml was co-incubated in BSA-MGO mixture (Fig. 2d). WMSE inhibitory potential was comparable to AG standard drug (Fig. 2c).

3.2.3. N-Acetylglycyl-lysine methyl ester (G.K.) peptide-ribose assay (last stage glycation)

GK peptide incubated with ribose showed an increased in fluorescence intensity due to protein cross linking. As seen in Fig. 1C, WMSE significantly reduced the intensity of the fluorescence, indicating anti-cross-linking activity. Our finding illustrates WMSE resilience against late glycation by-products.

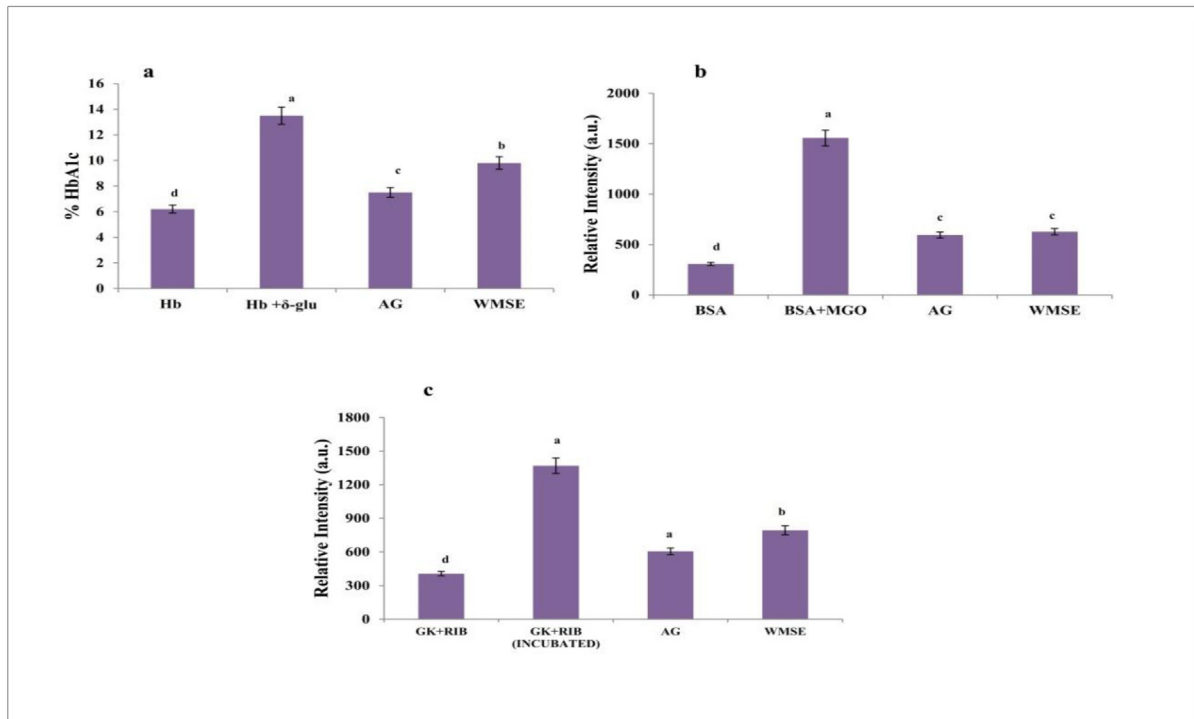


Fig. 1. Inhibition of AGEs formation. a) Early stage of glycation b) Intermediate stage of glycation c) Last stage of glycation. According to Tukey's HSD, bars with the same letters are not significantly different ($p \leq 0.05$). Hb: Hemoglobin, δ -glu: δ -gluconolactone, BSA: Bovine serum albumin, MGO: Methylglyoxal, GK: N-Acetylglycyl-L-lysine methyl ester (G.K.) peptide, RIB: Ribose, AG: Aminoguanidine (10 mM), WMSE: Watermelon seed extract (300 μ g).

4. Discussion

We demonstrated the antioxidant activities of WMSE extracts (inhibiting free radical activity) as well as their anti-glycation potential. Indeed, the present investigation demonstrated that the WMSE extracts may chelate transition metals, which may reduce antioxidative glycation and glycoxidation events, may be responsible for the protection against glycation-induced cross-linking. Many antioxidant-rich extracts have reportedly been shown to inhibit the production of AGEs by scavenging free radicals produced by the glycation process and blocking the

self-oxidation of reducing sugars and Amadori products [10]. Further, plant extracts are thought to prevent the production of AGEs because of their capacity to chelate metal ions [11]. In our study WMSE efficiently prevented the development of ferrous and ferrozine complexes, indicating that it had a chelating impact on ferrous ions before the combination with ferrozine was formed. This is most likely because of the phenolic and flavonoid content, which is one of the core components in metal ion chelation. It has been reported that the methanolic extract of watermelon seed contains phytochemical

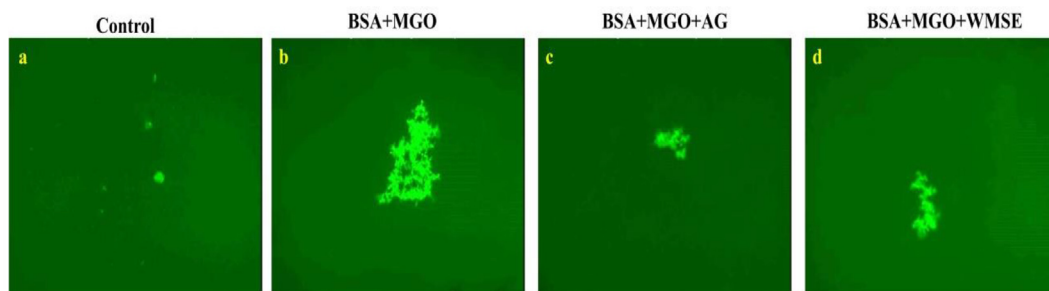


Fig. 2. Inhibitory effect of WMSE on MGO induced protein aggregation. BSA: Bovine serum albumin, MGO: Methylglyoxal, AG: Aminoguanidine (10 mM), WMSE: Watermelon seed extract (300 μ g).

components such as phenols and flavonoids, which possess good antioxidant activity.

The hemoglobin of RBCs reacts with δ -gluconolactone resulting in a significant increase in % HbA1c levels was observed. However, upon co-incubation with WMSE the % of HbA1c was gradually decreased. The majority of protein glycation in diabetes occurs at intra-chain lysine residues, producing early stage Amadori products that are later transformed into AGEs. Our research study led us to make the assumption that phytochemicals content in WMSE prevent the development of Amadori products because they can bind to free lysine amino groups of haemoglobin proteins. This prevents δ -gluconolactone reducing sugars from attaching to the amino groups of haemoglobin and thus prevents the formation of Amadori products. Our findings is agreement with Gutierrez et al. reported that *origanum majorana* had a direct impact on glycated haemoglobin production, probably due to its antioxidative action [12].

The BSA-MGO system is used to explore the prevention of the intermediate step of glycation, which produces reactive carbonyl species (RCS), specifically methylglyoxal and glyoxal, which then interact with long lived proteins. Thus, a dicarbonyl compound's reaction with proteins resembles the intermediate stage of protein glycation [13]. It was observed BSA incubated with MGO increased the fluorescence intensity due to AGEs formation. The presence of WMSE decreased the fluorescence intensity indicating inhibition of AGEs formation. These findings confirm the preventative impact of WMSE on glycation-induced aggregation and fibrillation of albumin and are in good agreement with the BSA-MGO assays by inhibiting Amadori product formation and trapping reactive dicarbonyl compounds. Our findings support Wu et al. that epicatechin gallate inhibits the carbonylation of BSA, the formation of amyloid cross-structures, and the development of AGEs using a BSA/fructose model [14].

Ribose was treated with a synthetic peptide called the G.K. peptide for 24 h. It was anticipated that this approach would produce peptides with advanced Maillard reaction products that would dimerize as a result of lysine–lysine cross-linking [15]. The main outcomes of the Maillard reaction are the development of fluorescence and protein cross-linking. WMSE exhibited anti cross-linking activity by decreasing fluorescence intensity, when compared to GK peptide in the presence of ribose. This finding can be explained by the fact that WMSE is rich in glycosidic compounds, especially saponin, which may have anticross-linking capabilities. Our findings concur with those of Xi et al. who found that saponin was most prevalent in the WMSE content.

Furthermore, saponin was also found to significantly block both early and late glycation of proteins. Antioxidant and anti-glycation effects were related to the presence of saponins [16].

5. Conclusion

WMSE has a significant inhibitory effect on protein glycation and oxidation-dependent protein damage. Furthermore, the findings suggest that WMSE works as a free radical scavenger as well as a metal ion chelating agent, preventing the formation of AGEs. The WMSE may assist to protect against hyperglycemia-mediated protein damage, which could be effective in the treatment of diabetes-related comorbidities. Our study provides preclinical evidence for the anti-glycation potential and safety of the WMSE, emphasising the necessity of clinical study in diabetic subjects to validate the WMSE as an adjuvant in the management of diabetes.

Financial support and sponsorship

The author(s) received no financial support for the current research.

Conflicts of interest

There are no conflicts of interest.

Acknowledgment

The authors respectfully thank everyone who helped to make this research achievable.

References

- [1] Singh VP, Bali A, Singh N, Jaggi AS. Advanced glycation end products and diabetic complications. *Korean J Physiol Pharmacol* 2014;18:1–14.
- [2] Velichkova S, Foubert K, Pieters L. Natural products as a source of inspiration for novel inhibitors of advanced glycation endproducts (AGEs) formation. *Planta Med* 2021;87: 780–801.
- [3] Ramazan A, Ozcan MM, Kanbur GS, Dursun N. Some physico-chemical properties of edible and forage watermelon seeds. *Iran. J. Chem. Chem. Eng* 2012;31:41–7.
- [4] Omre PK, Shikhangi Singh S. Waste utilization of fruits and vegetables–A review. *South Asian J Food Tech and Env* 2018; 4:605–15.
- [5] Salgado JM, Rodrigues BS, Donado-Pestana CM, Dos Santos Dias CT, Morzelle MC. Cupuassu (*Theobroma grandiflorum*) peel as potential source of dietary fiber and phytochemicals in whole-bread preparations. *Plant Foods Hum Nutr* 2011;66: 384–90.
- [6] Seidu KT, Otutu OL. Phytochemical composition and radical scavenging activities of watermelon (*Citrullus lanatus*) seed constituents. *Croat J Food Sci Technol* 2016;20:83–9.
- [7] Amran MA, Palaniveloo K, Fauzi R, Satar NM, Mohidin TB, Mohan G, Razak SA, Arunasalam M, Nagappan T, Sathiyaseelan JS. Value-added metabolites from agricultural waste

- and application of green extraction techniques. *Sustainability* 2021;13:1–27.
- [8] Chew YL, Goh JK, Lim YY. Assessment of in vitro antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia. *Food Chem* 2009;116:13–8.
- [9] Anandan S, Urooj A. Bioactive compounds from *Morus indica* as inhibitors of advanced glycation end products. *Indian J Pharmaceut Sci* 2019;81:282–92.
- [10] Elosta A, Ghous T, Ahmed N. Natural Products as Anti-glycation Agents: Possible Therapeutic Potential for Diabetic Complications. *Curr Diabetes Rev* 2012;8:92–108.
- [11] Younus H, Anwar S. Prevention of non-enzymatic glycosylation (glycation): Implication in the treatment of diabetic complication. *Int J Health Sci* 2016;10:261–77.
- [12] Gutierrez RM. Inhibition of advanced glycation end-product formation by *origanum majorana* L. in vitro and in streptozotocin-induced diabetic rats. *Evid Based Complement Alternat Med* 2012:1–8.
- [13] Nemet I, Varga Defterdarović L, Turk Z. Methylglyoxal in food and living organisms. *Mol Nutr Food Res* 2006;50:1105–17.
- [14] Wu X, Zhang G, Hu X, Pan J, Liao Y, Ding H. Inhibitory effect of epicatechin gallate on protein glycation. *Food Res Int* 2019;122:230–40.
- [15] Nagaraj RH, Shipanova IN, Faust FM. Protein cross-linking by the Maillard reaction isolation, characterization, and in vivo detection of a lysine-lysine cross-link derived from methylglyoxal. *J Biol Chem* 1996;271:19338–45.
- [16] Xi M, Hai C, Tang H, Chen M, Fang K, Liang X. Antioxidant and antiglycation properties of total saponins extracted from traditional Chinese medicine used to treat diabetes mellitus. *Phytother Res* 2008;22:228–37.