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Thiocolchicoside Ameliorated Glycation via Lysine Blockade and Carbonyl Entrapment

(Thiocolchicoside Diperbaharui Glisasi melalui Sekatan Lisin dan Perangkap Karbonil)

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ABSTRACT

The phenomenon of glycation leads to formation of AGE, which plays central role in various health hazards and accelerates the aging process. The re-purposing approach provide rapid means to introducing potential leads to drug discovery program. Keeping this into account, the present study investigates thiocolchicoside, for its capacity to be repurposed as anti-glycation agent. To assess the anti-glycation potential, thiocolchicoside was selected on the basis SAR, it is a muscle relaxant which is synthetic derivative of colchicoside, thiocolchicoside at 0.5, 1 and 2 mM were used and tested on various assays such as AGE inhibition assay (intrinsic fluorescence), fructosamine adduct formation (NBT assay) and availability of free lysine by using TNBSA followed by Lysine blockade assay (OPA and molecular docking study). The structural changes in the BSA protein was determined by using the thioflavin-T and Congo red assays. Finally, the carbonyl entrapment assay also performed to confirm the mechanism of the anti-glycation action. Thiocolchicoside significantly reduced the AGEs production in BSA-fructose model with an IC_{50} value of 0.25 mM. The fructosamine adducts were found to be reduced along with enhanced availability of free lysine. Furthermore, it exhibited lysine blockade activity which was also validated by computational study. Thiocolchicoside also prevented the alteration in glycation mediated BSA conformation. Furthermore, it was also found to entrap carbonyl moieties. Thiocolchicoside has a showed the significant anti-glycation potential, which can be attributed to its ability to block lysine residue and entrap carbonyl compounds. Hence, this clinically used muscle relaxant present itself as a potential drug to be repurposed as anti-glycation agent.

Keywords: BSA; glycation; HPLC; repurposing; thiocolchicoside

ABSTRACT

Fenomena glikasi membawa kepada pembentukan AGE, yang memainkan peranan utama dalam pelbagai bahaya kesihatan dan mempercepatkan proses penuaan. Pendekatan guna semula menyediakan cara pantas untuk memperkenalkan potensi petunjuk kepada program penemuan dadah. Dengan mengambil kira perkara ini, penyelidikan ini mengkaji thiocolchicoside untuk kapasitinya untuk digunakan semula sebagai agen anti-glikasi. Untuk menilai potensi anti-glikasi, thiocolchicoside telah dipilih berdasarkan SAR, ia adalah relaksan otot yang merupakan terbitan sintetik colchicoside, thiocolchicoside pada 0.5, 1 dan 2 mM telah digunakan dan diuji pada pelbagai ujian seperti asai perencatan AGE (intrinsik pendarflour) dan pembentukan fruktosamin secara bebas oleh aduk. TNBSA diikuti dengan asai sekatan lisin (OPA dan kajian dok molekul). Perubahan struktur dalam protein BSA ditentukan dengan menggunakan thioflavin-T dan ujian merah Congo. Akhir sekali, ujian perangkap karbonil juga dilakukan untuk mengesahkan mekanisme tindakan anti-glikasi. Thiocolchicoside telah mengurangkan pengeluaran AGEs dengan ketara dalam model BSA-fruktosa dengan nilai IC_{50} sebanyak 0.25 mM. Tambahan fruktosamin didapati berkurangan bersama-sama dengan peningkatan ketersediaan lisin bebas. Tambahan pula, ia menunjukkan aktiviti sekatan lisin yang juga disahkan oleh kajian pengiraan. Thiocolchicoside juga menghalang perubahan dalam konformasi BSA pengantara glikasi. Tambahan pula, ia juga didapati memerangkap bahagian karbonil. Thiocolchicoside mempunyai potensi anti-glikasi yang ketara yang boleh dikaitkan dengan keupayaannya untuk menyekat sisa lisin dan memerangkap sebatian karbonil. Oleh itu, pelemas otot yang digunakan secara klinikal ini menunjukkan dirinya sebagai ubat yang berpotensi untuk digunakan semula sebagai agen anti-glikasi.

Kata kunci: BSA; glikasi; guna semula; HPLC; thiocolchicoside

INTRODUCTION

Ageing, the progressive decline in tissue and organ function over time is a natural phenomenon inherent to human (Emel'yanov 2017). The non-enzymatic glycation theory highlights a direct correlation between the ageing and the accumulation of advanced glycation end products (AGEs), which primarily drive the decline in organ, tissue, and cellular functions (Khan et al. 2021; Monnier 1989). Glycation is a non-enzymatic reaction between carbohydrate carbonyl groups and protein amine groups particularly the N-terminal amino group and the side chains of arginine and lysine (Akhtar et al. 2017). The process induces conformational changes in proteins structure resulting in functional loss and aggregation which contribute to the onset of various disorders (Choi & Gandhi 2018). Major health issues include those related to diabetes (Abbas et al. 2016), neuropathies and cognitive impairment (Chowdhury et al. 2018), renal insufficiency (Saito & Marumo 2015), and hepatic insufficiency (da Silva Pereira et al. 2017). Beyond altering the proteins structures, AGEs bind to their receptors (called RAGEs), inducing glycative stress (Jakubczyk et al. 2020).

Drug repurposing methods for identifying the new therapeutic indication for existing drugs is widely employed today (Baker et al. 2018). This approach saves time and reduces financial costs. Regarding anti-glycation activity, functional groups as sulfhydryl and thiomethyl play vital roles in antiglycation action (Ahmed et al. 2005; Mil et al. 2021). Currently, no effective medicine exists for glycation. Although drugs such as aminoguanidine, pyridoxamine and benfotiamine have been screened for their anti-glycation potential they exhibit adverse effects, limited clinical efficacy, or non-specificity (Alshanwani et al. 2022). Consequently, this study was designed to identify and evaluate anti-glycation lead molecules using SAR analysis, *in-silico* computational studies and drug repurposing.

Thiocolchicoside, a muscle relaxant and synthetic sulphur-containing derivative of colchicoside, contains methylthio functional group in its chemical structure, making it a candidate for screening as a potential anti-glycation lead molecule (Umarkar, Bavaskar & Yewale 2011). This study aims to identify and evaluate an effective lead molecule for repurposing in anti-glycation drug discovery.

MATERIALS AND METHODS

CHEMICALS

The following chemicals were used in the study: 2,4,6-trinitrobenzenesulfonic acid (TNBSA), acetic acid, D-fructose, formic acid, nitroblue tetrazolium (NBT), *O*-phenyldiamine, phosphate buffer saline, sodium azide, sodium carbonate, sodium dihydrogen phosphate, sodium hydrogen phosphate, aminoguanidine (AG), thioflavin T (ThT), congo red (CR), bovine serum albumin (BSA), sodium dodecyl sulphate (SDS), 2-methylquinoxaline (2-MQ), and *O*-phthaldialdehyde 98% (OPA) sodium bicarbonate were obtained from Sigma Aldrich, (St. Louis, Missouri, USA). Dimethyl sulfoxide, ethanol and methanol were obtained from Merck, New Jersey, USA. Thiocolchicoside was obtained from Sanofi (Karachi, Pakistan).

ANTIGLYCATION ASSAY

This assay was performed as previously described by Núñez et al. (2023). D-fructose (100 mM) and BSA (10 mg/mL) were mixed in phosphate buffer (0.2 M, pH 7.4) containing sodium azide (0.1%). The reaction mixtures contained either native BSA, glycated BSA (BSA + Fructose) or glycated BSA with or without standard aminoguanidine (5 mM) and test substance thiocolchicoside (0.5, 1, or 2 mM). These samples were incubated at 60 °C for 24 h. Subsequently, 200 µL aliquots were transferred in triplicate into 96 well black plates to determine the fluorescence intensity (excitation: 360/40 nm, emission: 460/40 nm) using a JASCO spectrofluorometer (Hachioji, Tokyo, Japan).

FRUCTOSAMINE ADDUCT ASSAY

This assay, based on the reaction mixtures of the anti-glycation assay using NBT, was performed as previously described (Arfat et al. 2014). BSA (100 µL), either native, glycated with aminoguanidine or with thiocolchicoside was mixed with carbonate buffer (100 mM, 1000 µL and 10.35 pH) containing NBT (0.25 mM). The samples were incubated in the dark for 2 h and 200 µL aliquots were transferred to 96-well microplates for absorbance measurement (525 nm) using a JASCO spectrophotometer (Hachioji, Tokyo, Japan).

TNBSA ASSAY

The assay was performed on reaction mixtures of anti-glycation assay using 2,4,6-trinitrobenzenesulfonic acid (TNBSA) as described earlier (Khan & Naseem 2023), Briefly, 500 µL reaction mixture were mixed with TNBSA solution (0.1% w/v, 250 µL) and incubated for 2 h. Absorbance was then measured at 335 nm using a JASCO spectrophotometer (Hachioji, Tokyo, Japan).

CIRCULAR DICHORISM

The secondary structure of BSA in the reaction mixture was analysed using circular dichorism (CD) a JASCO J810 Spectropolarimeter (Hachioji, Tokyo, Japan). At 25 °C temperature, glycated protein samples were scanned at least four times in the UV (250-400 nm) and far-UV amide (190-250 nm) to obtain the spectra. The CD spectral analysis was performed using DichroWeb server to determine secondary structural alterations in the protein (Gil et al. 2000).

LYSINE BLOCKADE ASSAY

The lysine blockade assay was performed using OPA method as previously described (Goodno, Swaisgood & Catignani 1981; Zenker et al. 2020). To prepare the OPA reagent (100 mL), OPA (80 mg) was dissolved in ethanol (95%, 2 mL), combined with β-mercapethanol (200 uL), 5 mL of 20% (w/w) sodium dodecyl sulfate (SDS) and 50 mL of 0.1 M sodium tetraborate buffer (pH:10). The reagent was stored in a dark airtight container. This reagent loses 5-10% of its stability daily, making it stable for only few days. The free lysine residue was measured fluorometrically by dissolving 25 µg of protein in de-ionized water (50 mL) in a vial containing freshly prepared OPA reagent (3 mL). 2 min after the reaction, measurements were taken using a spectrofluorometer (JASCO Hachioji, Tokyo, Japan) with excitation at 360/40 nm and emission at 460/40 nm.

in silico STUDIES

The 3D crystal structure of BSA (PBB ID: 4F5S) was retrieved by protein data bank. The 2D structures of the ligand, Thiocolchicoside was downloaded by using PUBCHEM as SDF files and that were transformed to 3D format by PYMOL 3.11, respectively (Nguyen et al. 2021). Finally, the target molecular docking studies was performed by using Auto dock vina 1.1.2 version. Both the Prepared Receptor and ligands were transformed to 'pdbqt' format, respectively, for the docking. The active sites were predicted by Prank web database and the grid was set as x = 100, y = 100 and z = 100 along with the coordinate sizes x= 4.368, y = 16.991 and z = 106.819, respectively. Once docking was completed, the most stable confirmation of ligand-protein interaction was used for the analysis of docking results with the help of Biovia Discovery tool 2021 client (Trott & Olson 2010).

THIOFLAVIN-T ASSAY

The ThT stock solution (100 µM) was prepared in phosphate buffer (pH 7.3) and kept in the dark, where it remained stable for a week. A 20 µM working solution (160 µL) was mixed with 40 µL of each protein sample from reaction mixtures incubate for 60 min. Following that, each sample (200 µL in triplicate) was transferred to 96 well- black plates for fluorescence measurement (excitation at 460 nm and emission at 485 nm) using a spectrofluorophotometer (JASCO, Hachioji, Tokyo, Japan) as described earlier (Waseem et al. 2023).

CONGO RED ASSAY

A 0.4 µM Congo red stock solution was prepared in Tris buffer (pH 7.3) and mixed with samples (40 mL) from each reaction mixture (160 µL). After 20 min at 25 °C, the absorbance was measured at 530 nm using a JASCO spectrophotometer (Hachioji, Tokyo, Japan) as previously described (AL-SAEDI et al. 2023).

CARBONYL ENTRAPMENT ASSAY

The carbonyl entrapping assay was performed using HPLC as previously described (Liu et al. 2018). Briefly, 0.4 mg/mL MGO was prepared using 0.1 M sodium phosphate buffer (pH 7.4). OPD (10.8 mg/mL) was dissolved in pure methanol. Two different vials were filled with 50% methanol (1 mg/mL) with 5-MQ (internal standard) and 2-MQ, respectively. Aminoguanidine (positive control) was prepared by dissolving 0.1 mg/mL in a 0.1 M PBS buffer (pH 7.4). A negative control was prepared by dissolving 50 uL of 5-MQ and MGO in PBS (850 uL). Thiocolchicoside (0.5 mM, 1 mM and 2 mM) was prepared. After mixing, the samples were incubated at 60 °C for 24 h. Thereafter, OPD solution (200 uL) was added to each vial and vortexed for 5 s. The samples were incubated in the dark for 30-min to allow derivatisation. The MGO content was quantified based on the amount of 2-MQ in each sample. HPLC analysis was performed using Prominence system (Shimadzu, Kyoto, Japan). The mobile phase consisted of 50% methanol and 5% glacial acetic acid, with a flow rate of 0.5 mL/min. All samples were filtered using a 0.22 µm syringe filter before injecting 10 µL into the HPLC system. The analysis ran for 30 min. Unreacted MGO was determined by comparing the 2MQ and 5MQ ratios and outcomes to that of the control (Ni et al. 2021).

STATISTICAL ANALYSIS

Data are presented as mean ± SEM (n=3). Statistical analysis was conducted using one-way ANOVA followed by post hoc Least Significant Difference analysis using SPSS (version 20.0, IBM-SPSS, USA). Significance levels were set at $p<0.05$, $p<0.0$, and $p<0.005$ compared to the respective controls.

RESULTS

in-vitro ANTIGLYCATION ASSAY

The glycated BSA (gBSA) exhibited significant ($p<0.005$) increase in fluorescence intensity as compared to control i.e., native BSA (nBSA) (Figure 1). The treatment groups i.e., standard Aminoguanidine (AG, 5mM) and test Thiocolchicoside (Thio, 0.5 mM, 1 mM and 2 mM) caused significant decline (IC_{50} ~ 0.25 mM) in the fluorescence intensity ($p<0.005$) as compared to gBSA.

FRUCTOSAMINE ADDUCT ASSAY

The gBSA has shown significantly ($p<0.005$) increased absorbance as compared with the nBSA (Figure 2). The AG treatment has significantly reduced the absorbance in comparison with gBSA. In case of Thio treatment, the decrease in absorbance was noted at all doses, which was significant only at lowest tested dose of 0.5 mM.

TNBSA ASSAY

The gBSA group exhibited significant ($p<0.005$) decrease in the absorbance as compared with nBSA (Figure 3). The AG (5 mM) and Thio (0.5 mM, 1 mM and 2 mM) treatments has significantly ($p<0.005$) increased the absorbance as compared to gBSA.

CIRCULAR DICHORISM

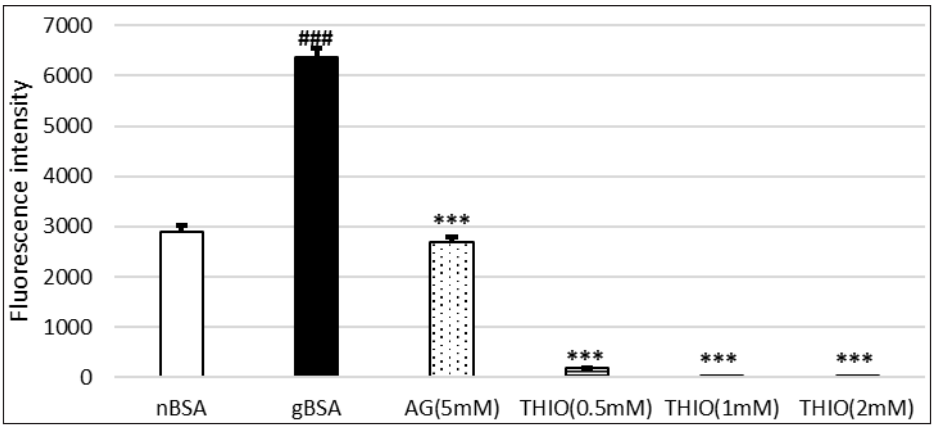
The CD spectra showed a glycation-induced shift in the secondary structure of BSA from alpha-helical to beta sheets (Figure 4). Thio and aminoguanidine both preserved the natural structure of the BSA.

LYSINE BLOCKADE ASSAY

The AG (5 mM) showed slight increase in the fluorescence intensity as compared with nBSA. However, the Thio treatment (1 mM and 2 mM) caused the significant ($p<0.005$) decline in the fluorescence intensity as of free lysine availability as compared to gBSA (gBSA, Figure 5).

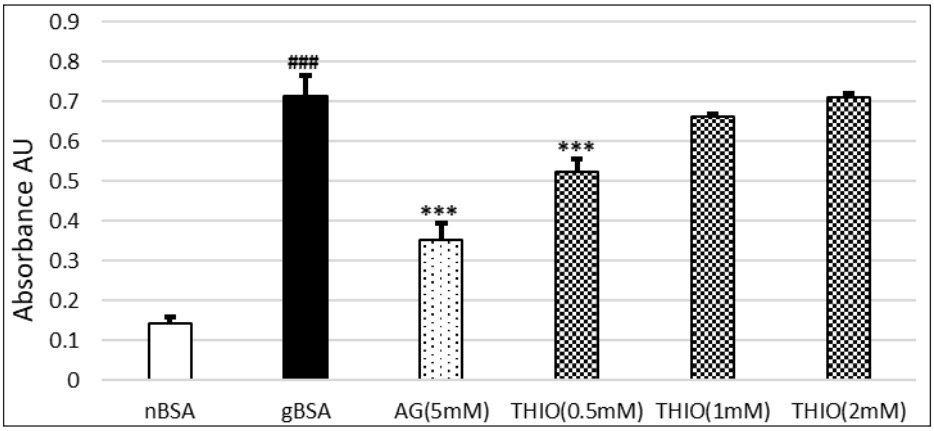
in-silico STUDY

Molecular docking interaction studies was performed between thiocolchicoside and BSA (Figure 6). The thiocolchicoside interacted with various amino acids (LYS, LEU, TYR, VAL, ASP, CYS, PHE, MET, ASN, GLY) especially the catalytic residue (LYS-524) involved in mediating the glycation reaction.



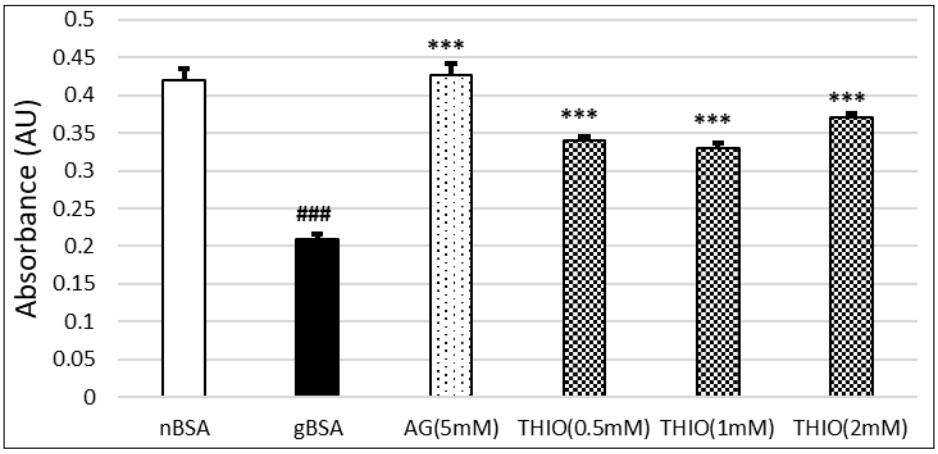
The figure shows the increased fluorescense intensity in the gBSA as compared with nBSA. The AG (5 mM) and Thio (0.5 mM, 1 mM and 2 mM) exhibited the significant decline in the flourescense intensity as compared to gBSA. The signs i.e., hash (#) and asterisk (*) shows statistical comparison with native and glycated BSA, respectively. The results are provided as mean ± SEM of fluorescence (indicative of AGEs intrinsic fluoresence) intensity (n=3)

FIGURE 1. Effect of thiocolchicoside on antiglycation assay



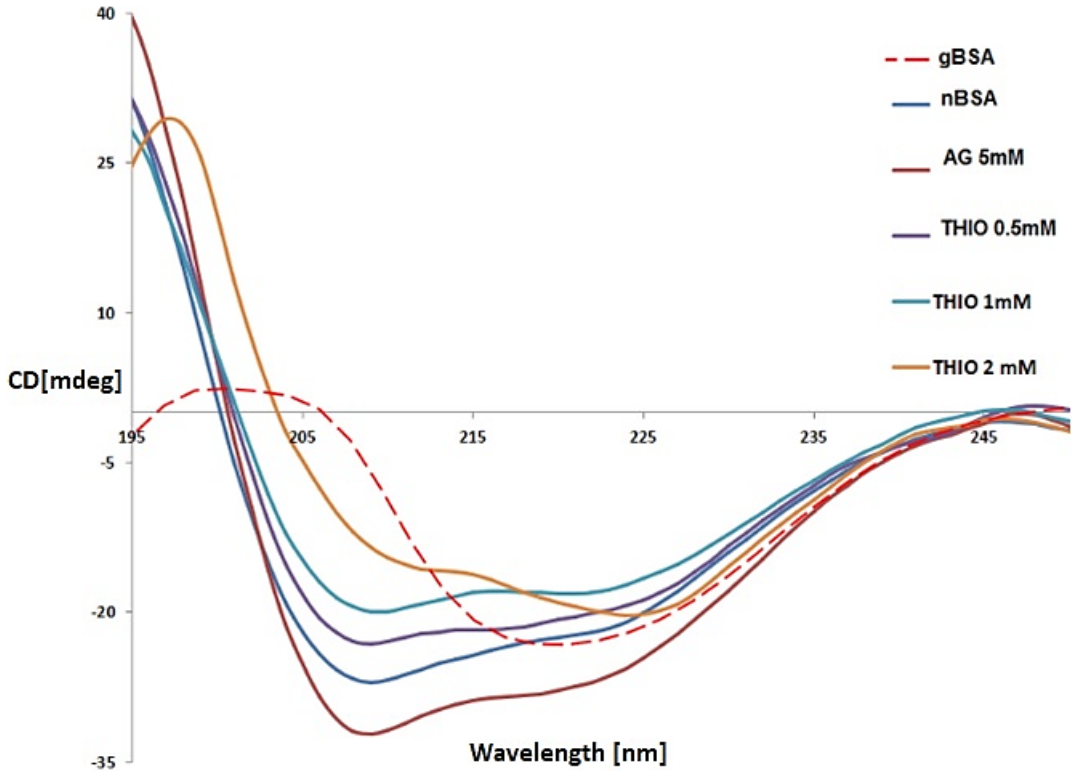
The figure shows mean ± SEM of the fructosamine content as absorbance (n=3). The gBSA group shows significant increase in absorbance as compared with nBSA. The AG (5 mM) and Thio (0.5 mM) exhibited the significant decline in the abosrbance as compared with gBSA. The signs i.e., hash (#) and asterisk (*) shows statistical comparison with native and glycated BSA, respectively

FIGURE 2. Effect of thiocolchicoside on fructosamine adduct assay



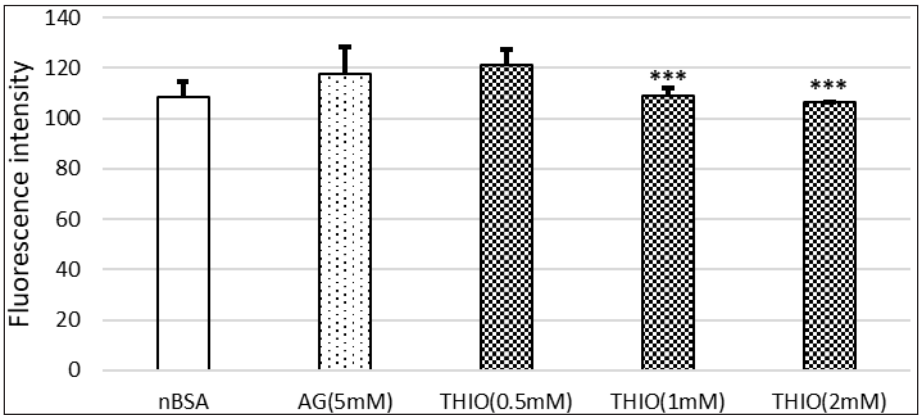
The figure shows the significant decrease in absorbance in the gBSA group as compared with nBSA. Meanwhile, the treatment groups AG (5 mM) and Thio (0.5 mM, 1 mM and 2 mM) significantly increased the absorbance as compared with gBSA. The signs i.e., hash **(#)** and asterisk (*) shows statistical comparison with native and glycated BSA, respectively. The data is represented as mean ± SEM of absorbance in TNBSA assay (n=3)

FIGURE 3. Effect of thiocolchicoside on TNBSA assay



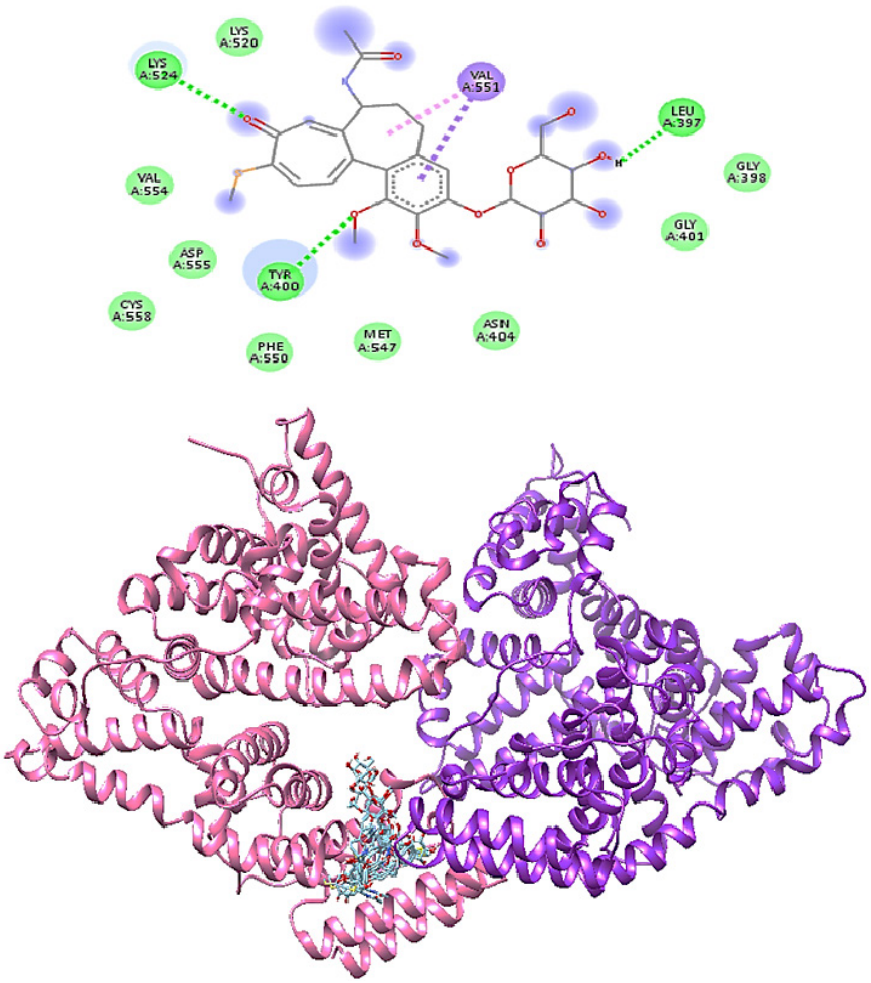
The figure depicts that the glycation has drastically altered the secondary structure of native BSA from alpha-helical to beta sheets. However, Thio (0.5 mM, 1 mM, and 2 mM) and Aminoguanidine prevented this alteration

FIGURE 4. Effect of thiocolchicoside on the conformation of BSA using Far-UV CD spectra



The figure depicts mean ± SEM of the free lysine availability as fluorescence intesity (n=3). The AG (5 mM) caused non-significant increase in fluorescence intensity as compared to nBSA. The Thio treatment exhibited significant decline in the fluorescence intensity at highest doses (1 mM and 2 mM) as compared to nBSA. The signs i.e., hash (#) and asterisk (*) shows statistical comparison with native and glycated BSA, respectively

FIGURE 5. Effect of thiocolchicoside on OPA assay for lysine blocking activity



The figure depicts the interaction of BSA (PBB ID: 4F5S) with THIO obtained using *in-silico* study. THIO was found to engage various residues of BSA i.e., LYS, LEU, TYR, VAL, ASP, CYS, PHE, MET, ASN, and GLY

FIGURE 6. Binding Interaction of thiocolchicoside with different residues of BSA

THIOFLAVIN T ASSAY

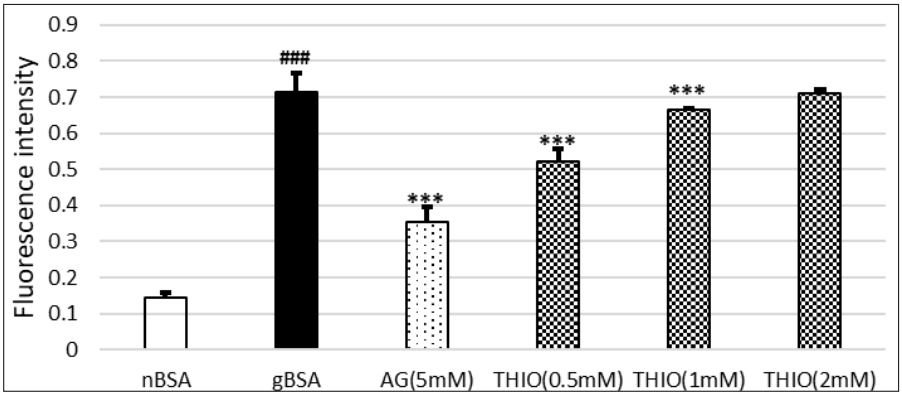
The gBSA group showed significant ($p< 0.005$) increase in fluorescence intensity as compared to nBSA (Figure 7). The AG (5 mM) has significantly decreased in the fluorescence as compared to gBSA. The thiocolchicoside treatment also caused decrease in fluorescence which became non-significant at highest tested dose of 2 mM.

CONGO RED ASSAY

The gBSA group caused significant ($p<0.005$) elevation in absorbance as compared with nBSA (nBSA, Figure 8). The AG (5 mM) and the Thio (0.5 mM, 1 mM and 2 mM) treatments showed significantly decline in the fluorescence as compared to gBSA.

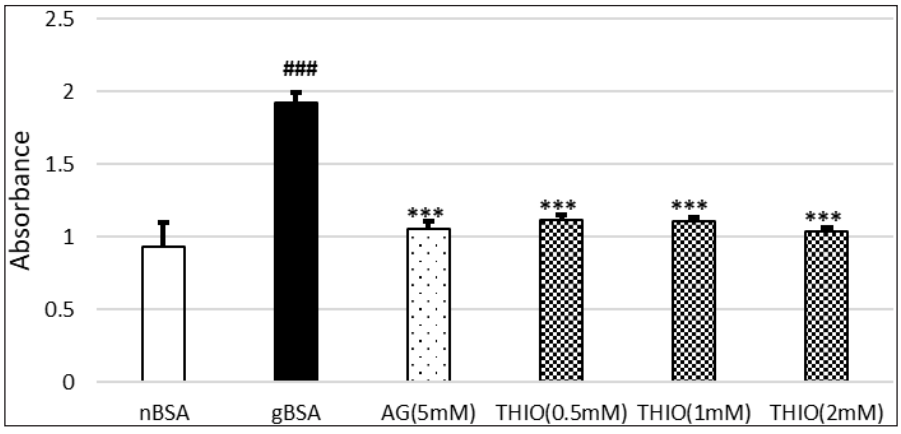
CARBONYL ENTRAPPING ASSAY

The AG and THIO (0.5, 1 and 2 mM) treatment caused significant decrease in the area under the curve (AUC) of 2-MQ. The aminoguanidine showed the 52% inhibition in formation of 2-MQ while thiocolchicoside also demonstrated carbonyl entrapment by approximately 90%. The representative chromatograms of aforementioned treatments are present in Figure 9.



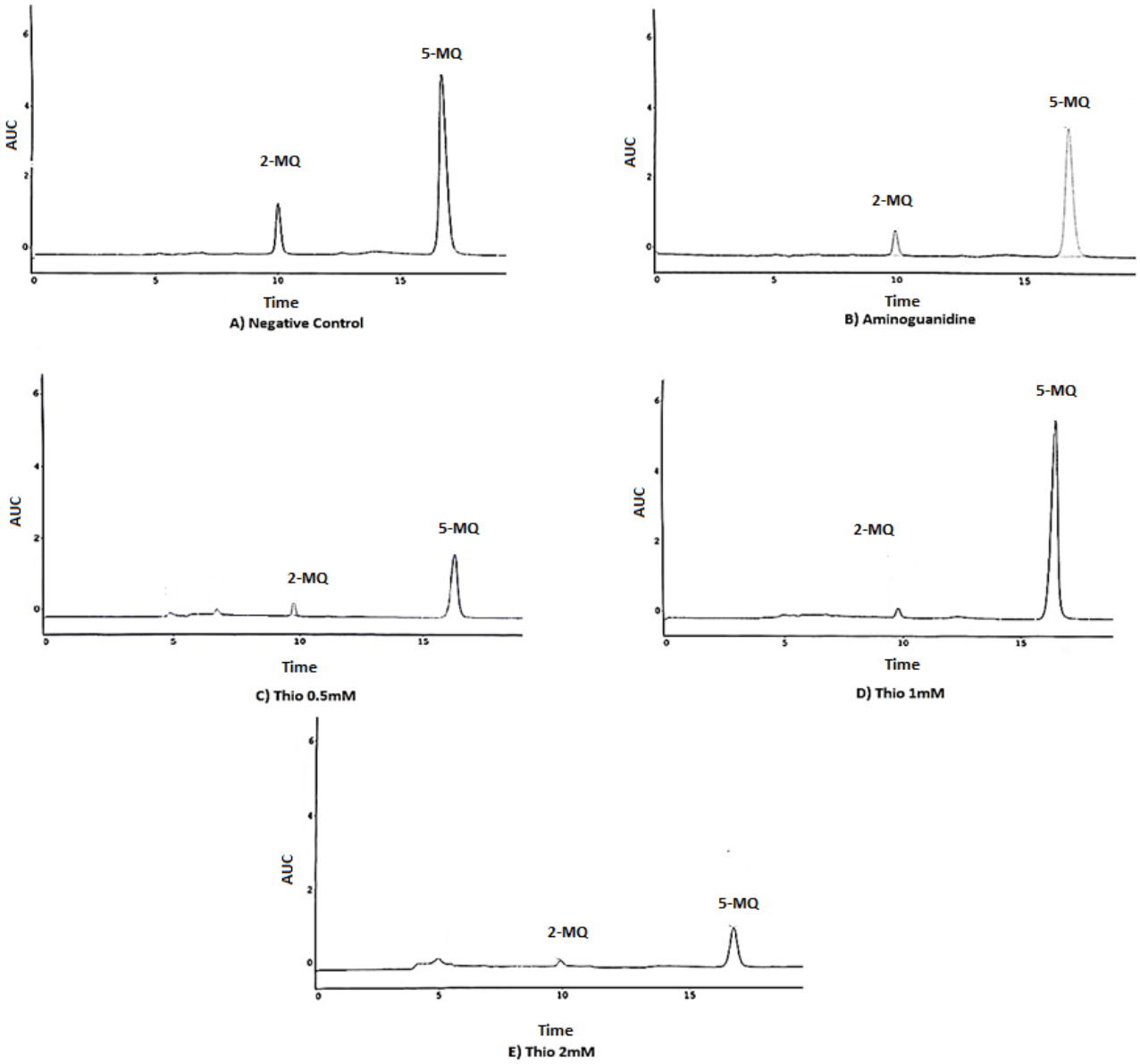
The figure depicts mean ± SEM of the ThT fluorescence (n=3). The gBSA group showed significant increase in the fluorescence intensity as compared to nBSA. However, the AG (5 mM) caused significant decline in fluorescence as compared to gBSA. The Thio treatment (0.5 mM and 1 mM) also exhibited the decrease in fluorescence which was non-significant at highested tested dose. The signs i.e., hash (#) and asterisk (*) shows statistical comparison with native and glycated BSA, respectively

FIGURE 7. Effect of thiocolchicoside on thioflavin T assay



The figure depicts mean ± SEM of the absorbance due to CR binding (n=3). The gBSA group showed the significant increase in fluorescence intensity as compared with nBSA. Whereas, the AG (5 mM) caused significant decrease in fluorescence intensity as compared to gBSA. The Thio treatment (0.5 mM, 1 mM, 2 mM) exhibited significantly decline in the fluorescence intensity as compared to gBSA. The signs i.e., hash (#) and asterisk (*) shows statistical comparison with native and glycated BSA, respectively

FIGURE 8. Effect of thiocolchicoside on Congo red assay



The figure depicts the representative chromatograms of carbonyl entrapping assay in the presence of AG and THIO. The 2-MQ peak was found in the negative control group while it was significantly reduced following AG and THIO treatments. The percent change of aminoguanidine was found to be 52%, whereas Thiocolchicoside treatments (0.5, 1 and 2 mM) caused inhibition of 98%, 78%, and 92%, respectively

FIGURE 9. Effect of thiocolchicoside on carbonyl entrapping assay

DISCUSSION

Glycation is primary mechanism underlying ageing and associated morbidities. Currently, no drug is available to counteract this harmful process. The repurposing offers a robust method for identifying lead molecules, which was the objective of this study (Gupta et al. 2018). Anti-glycation activity can be influenced by functional groups, such as thio (sulphur-containing) methoxy (–OCH_3), and methylthio (-SHC_3) groups (Mutha et al. 2019). Methoxy groups, particularly in aromatic rings, enhance antioxidant activity (Chen et al. 2020). Antioxidants counteract reactive oxygen species involved in glycation-induced oxidative stress, and methoxy-containing compounds may also scavenge reactive intermediates during glycation, slowing the process. Sulphur-containing groups exhibit nucleophilic properties, similar to thio groups. Their reactivity allows them to neutralizes reactive intermediates involved in glycation. These compounds can act as metal chelators, influencing metal-catalysed reactions (Mutlu et al. 2019). Given their chemical structure, drugs with sulphur containing functional groups such as thio or sulphahydryl groups are most likely to have anti-glycation potential. Based on these attributes, thiocolchicoside, a clinically used muscle relaxant, was selected for assessing the anti-glycation activity. The results show that thiocolchicoside significantly reduced AGE formation (Figure 1). Literature

suggests that compounds possessing sulphahydryl and thio groups can resist glycation (Ahmed et al. 2005; Mil et al. 2021). Based on these chemical properties, thiocolchicoside proved effective.

Glycation is a complex phenomenon involving a cascade of events that lead to AGE formation (Twarda-Clapa et al. 2022). Hence, the impact of thiocolchicoside on early glycation steps was assessed using the fructosamine adduct assay. The result indicates that only the lowest dose 0.5 mM reduced early glycation adducts formation (Figure 2). Hence, the early lysine-fructose interaction may be a key mechanism underlying the anti-glycation effect of thiocolchicoside (Parwani & Mandal 2023). Free lysine availability was assessed using the TNBSA assay. The results indicate significantly higher free lysine availability after thiocolchicoside treatment compared to glycated BSA (Figure 3). This suggests that thiocolchicoside interferes with the fructose-lysine interaction during the early step in the glycation process. Notably, no significant differences were observed among the tested doses indicating that the lowest dose to be most effective in ameliorating the glycation.

Glycation induces conformational changes in BSA, altering its secondary structure to amyloid-like or β-enriched sheets (Iannuzzi, Irace & Sirangelo 2014). The CD was used to assess the effect of thiocolchicoside on these glycation-induced structural modifications. Native BSA, rich in alpha helical structure showed a positive band at 192 nm and two negative bands at 208 nm and 222 nm. In contrast, glycated BSA exhibited a positive band around 195 nm and a unique negative band at 218 nm, indicating beta-sheet formation (Miles, Ramalli & Wallace 2022). Notably, treatments with aminoguanidine and thiocolchicoside yielded CD spectra identical to those of native BSA, indicating the retention of secondary structure (Figure 4).

To confirm the interference of thiocolchicoside with the fructose-lysine interaction, the lysine blockade assay was performed using OPA reagent (Yang et al. 2023). Consistent with previous literature, aminoguanidine did not block lysine, while thiocolchicoside significantly blocked it (Figure 5). Hence, the assay confirmed the lysine blocking ability of thiocolchicoside as potential anti-glycation mechanism. To confirm the ability of thiocolchicoside to interfere with fructose-lysine interactions, a computational study was performed. The docking results showed that thiocolchicoside binds to LYS 524 via hydrogen bonding (Figure 6). Study suggests LYS 524 in BSA plays a vital role in mediating the glycation reaction (Anwar et al. 2023). Thus, the computational results also validate that thiocolchicoside has lysine blockade potential.

BSA, a 66kDa protein is primarily α-helical in structure (Wu et al. 2018). Glycation causes to conformational changes and the formation of structured clumps that bear similarities to amyloid (Hampel et al. 2021). To assess these glycation induced amyloid-like aggregates ThT (Emendato et al. 2018) and CR (Oso, Olaoye & Oso 2023) assays were performed. The results show significantly increased aggregates in glycated BSA samples, which were reduced by thiocolchicoside, as indicated by ThT (Figure 7) and CR (Figure 8) assay. Notably, the lowest tested dose of 0.5 mM was most effective in reducing aggregate formation.

The carbonyl entrapment mechanism supports the of anti-glycation action (Yadav, Palkhede & Kim 2023). The aminoguanidine is a known carbonyl entrapper, which aligns with our results thereby validating our methodology (Figure 9). Our data indicating that thiocolchicoside significantly reduce AUC of 2MQ, suggesting carbonyl entrapping and inhibition of glycation progression.

A limitation of this study includes is utilization of a protein-sugar model to assess the anti-glycation potential of thiocolchicoside. This could be addressed by employing a multifaceted approach including multiple protein-sugar models, *in-vivo* studies and exploring glycation in multiple tissues in laboratory animals. Additionally, *in-vivo* experiments with laboratory animals are lacking.

CONCLUSION

This study demonstrates that thiocolchicoside inhibits glycation, blocking lysine residues in BSA and entrapping carbonyl intermediates making it a promising candidate for repurposing in anti-glycation drug discovery.

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