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Research Paper

Nanotechnology-Based Nanopolymeric Polyherbal Formulation for Enhanced Antioxidant and Anti-Glycation Activity

Febriana Astuti[1,3], Mustofa[2], Arif Budi Setianto[1], Akrom[1*]

[1]*Doctoral Study Program of Pharmacy Sciences, Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Daerah Istimewa Yogyakarta, 55164, Indonesia*
[2]*Faculty of Medicine, Public Health and Nursing, Gadjah Mada University, Yogyakarta, Daerah Istimewa Yogyakarta, 55281, Indonesia*
[3]*D3 Pharmacy Study Program, Politeknik Kesehatan TNI AU Adisutjipto, Yogyakarta, Daerah Istimewa Yogyakarta, 55198, Indonesia*
*__Corresponding author__: akrom@pharm.uad.ac.id

Abstract
Elevated oxidative stress and glycation give to the enhancement of degenerative condition such as diabetes and heart disease. Herbal extracts such as *Apium graveolens, Centella asiatica,* and *Orthosiphon stamineus* possess well-documented antioxidant and anti-glycation properties. However, their therapeutic effectiveness is measured by poor bioavailability, highlighting the need for innovative formulations. Nanotechnology presents a indicative of success approach by improving the solubility and distribution of active compounds. This study developed a nanopolymeric formulation by combining extracts of *Apium graveolens, Centella asiatica,* and *Orthosiphon stamineus* utilizing the ionic gelation method by chitosan, NaTPP, and Tween 80. The nanoparticles were characterized based on particle size, PDI, zeta capability, TEM, and FTIR. In vitro assays utilizing peripheral blood mononuclear cells (PBMCs) were conducted to analyze antioxidant activity through Nrf2 and GST expression, and anti-glycation capability by measuring AGEs. The nanopolyherbal particles had an average size of 186 ± 46.81 nm, by a PDI of 0.434 ± 0.04 and a zeta capability of -29.69 ± 1.54 mV. Both 1% and 3% nanopolyherbal treatments increased Nrf2 stages (66.77 ± 7.65 and 63.13 ± 2.75, respectively) and GST stages (87.97 ± 49.48 and 73.57 ± 4.61) compared to the control group (Nrf2: 54.1 ± 1.0; GST: 68.93 ± 2.28). The nanopolyherbal formulation also significantly decreased AGE itemion (1%: 1075.67 ± 107.51; 3%: 813.33 ± 117.05) compared to the control (1497.33 ± 161.58). These outcomes reveal that the nanopolyherbal formulation enhances antioxidant activity and inhibits glycation in PBMCs, suggesting its capability for managing oxidative stress-related condition.

Keywords
Antiglycation, Antioxidant, Chitosan-Sodium Tripolyphosphate, Nanopolymeric Polyherbal

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1. INTRODUCTION

Glycation is a process in that decreasing sugars react by proteins or lipids devoid of enzymatic involvement, outcoming in the irreversible construction of advanced glycation end items (AGEs) (Perrone et al., 2020). The concentration of advanced glycation end product (AGEs) in various tissues has been strongly associated by the enhancement of several chronic degenerative condition, comprising metabolic disorders such as diabetes mellitus; cardiovascular conditions such as atherosclerosis and chronic heart failure; neurodegenerative condition such as Alzheimer's; and progressive renal dysfunction. The molecular cross-linking properties of AGEs give to their deleterious effects on tissue structure and function across these diverse disease states (Hong Sheng et al., 2017). The excessive concentration of AGEs gives to oxidative stress, inflammation, and endothelial dysfunction, thereby exacerbating diabetes-related complications, particularly cardiovascular conditions such as heart failure, hypertension, and atherosclerosis. Consequently, inhibiting AGE construction represents a indicative of success strategy for preventing morbidities associated by oxidative stress and its related effects (Prasad, 2021).

Phytotherapeutic approaches give a biological strategy to reduce oxidative stress and inhibit the construction of AGEs, that are implicated in various pathological conditions. Among the botanicals receiving considerable scientific attention are *Apium graveolens, Centella asiatica,* and *Orthosiphon stamineus*, due to their performanced therapeutic capability. These plants contain a diverse array of bioactive phytochemicals that exhibit both antioxidant and anti-inflammatory effects, offering preventive benefits against chronic degenerative condition, particularly cardiovascular disorders. Additionally, their immunomodulatory properties, mediated through complex biochemical mechanisms, enhance their cytoprotective effects against ox-

idative damage and protein glycation.

The therapeutic effectiveness of apigenin-the primary bioactive flavonoid in *Apium graveolens*-has been scientifically validated through its ability to reduce the synthesis of AGEs via two mechanisms: (1) lowering oxidative stress by scavenging free radicals and (2) competitively inhibiting the binding of AGEs to the AGE-RAGE (receptor for advanced glycation end product) complex (Allemailem et al., 2024; Zhou et al., 2019). Parallel studies have performanced that asiaticoside, a triterpenoid saponin extracted from *Centella asiatica*, exhibits strong antioxidant properties by upregulating endogenous defense mechanisms through Nrf2 activation. Additionally, it modulates the enzymatic activity of nitric oxide synthase (NOS), thereby contributing to the maintenance of redox homeostasis (Wei et al., 2025; Zhu et al., 2020; Zweig et al., 2021). Sinensetin, found in *Orthosiphon stamineus*, has strong antioxidant, hepatoprotective, and immunomodulatory properties (Andira et al., 2024; Tabana et al., 2016). However, despite their indicative of success pharmacological benefits, the therapeutic application of these bioactive compounds is measured by poor bioavailability due to low solubility and permeability.

The bioactive compounds in *Apium graveolens, Centella asiatica,* and *Orthosiphon stamineus* exhibit measured absorption in living organisms due to their chemical structures. For instance, apigenin and sinensetin are lipophilic and have low water solubility, outcoming in decreased bioavailability (Chen et al., 2023b; Zhanga et al., 2024). Asiaticoside, on the other hand, is hydrophilic-readily dissolving in water-but exhibits measured permeability through lipophilic cell membranes, that hinders its ability to effectively reach target sites (Bandopadhyay et al., 2023). These solubility and permeability challenges significantly reduce the therapeutic effectiveness of these compounds.

Nanotechnology-based formulations offer a novel approach to overcoming these challenges. The use of nanopolymeric carrier systems enhances the dissolution, distribution, absorption, and overall efficacy of both lipophilic and hydrophilic bioactive compounds in the body. (Syahputra et al., 2024). Optimizing the particle size and surface characteristics of nanopolymeric formulations enables precise targeted delivery and sustained release. This strategy enhances therapeutic efficacy while simultaneously decreasing the required dosage (Ayala-Fuentes and Chavez-Santoscoy, 2021). Numerous studies have performanced that drug delivery systems utilizing nanoparticles significantly enhance the pharmacological effects of herbal extracts, outcoming in improved therapeutic outcomes.

Although numerous studies have investigated the antioxidant and anti-glycation properties of individual plant compounds or single herbal extracts, this research adopts a more comprehensive approach by combining extracts from *Apium graveolens, Centella asiatica,* and *Orthosiphon stamineus* into a single nanopolymeric formulation. This approach aims to overcome bioavailability limitations and enhance the synergistic effects of the bioactive constituents—benefits often unattainable when utilizing individual extracts. This study represents a significant advancement in the field of natural medicine, as it is the first to integrate these three specific plant extracts into a unified nanopolymeric system. This study aims to develop a novel combination of herbal extracts formulated as a nanopolymeric system and to rigorously analyze its efficacy in decreasing oxidative stress and preventing glucose-induced molecular damage. A comprehensive laboratory investigation will be conducted utilizing peripheral blood mononuclear cell (PBMC) cultures to elucidate the underlying mechanisms of the formulation's action, by particular emphasis on the modulation of the NRF2-Keap1-ARE signaling pathway and the enhancement of glutathione S-transferase (GST) activity. These processes play a critical framework in strengthening the body's endogenous antioxidant defenses and decreasing stages of harmful AGEs. Ultimately, this research aims to give to the advancement of nanotechnology-based polyherbal formulations designed to mitigate oxidative damage and glycation-related health complications.

2. EXPERIMENTAL SECTION

2.1 Materials

Leaves of *Apium graveolens, Centella asiatica,* and *Orthosiphon stamineus* were collected from Tawangmangu, Central Java, and subsequently identified at the Biology Learning Laboratory of Ahmad Dahlan University, Yogyakarta. Extraction was performed utilizing 70% ethanol as the solvent. The nanosuspension formulation included chitosan, sodium tripolyphosphate (NaTPP), Polysorbate 80, and acetic acid. Antioxidant and antiglycation activities will be analyzed in peripheral blood mononuclear cells (PBMCs) utilizing ELISA kits for NRF2, GST, and AGEs supplied by Biovendor.

2.2 Methods

2.2.1 Extract Preparation

Preparation of Extract as previously detailed by Siska et al. (2018) fresh leaves were desiccated in a tray oven at 50°C for 24 hours and subsequently pulverized utilizing a grinder. Leaf powders of *Apium graveolens* and *Orthosiphon stamineus* were macerated in 70% ethanol, while Centella asiatica was processed utilizing 96% ethanol. The maceration took place at room degree (25 ± 2°C) for 24 hours. Following filtration, the outcoming extracts were concentrated and dried utilizing a rotary evaporator (Buchi®R-300 Rotavapo).

2.2.2 Preparation of Nanopolyherbal

The preparation of nanopolyherbal formulations begins by combining solutions of chitosan, NaTPP, and extracts of *Apium graveolens, Centella asiatica,* and *Orthosiphon stamineus*. Each extract was precisely weighed to ensure accuracy: 150 mg of *Apium graveolens* extract, 300 mg of *Centella asiatica* extract, and 150 mg of *Orthosiphon stamineus* extract. Six grams of Tween 80 were measured. A 1% chitosan solution was then prepared by weighing 1 gram of chitosan powder and dissolving it in 100 mL of 1% acetic acid solution. The dissolution process was figured out by gently swirling by a magnetic stirrer until a

uniform solution was achieved. A 0.1% NaTPP solution was made by mixing 100 mg of sodium tripolyphosphate in 100 mL of distilled water and stirring it well until it completely dissolved (Mardiyanto et al., 2024). The preparation of the herbal nanoparticle begins by the combination of 150 mg of celery extract, 300 mg of pegagan extract, and 150 mg of cat's whiskers extract in 6 grams of Tween 80 solution. This mixture is gradually blended by a mortar until it becomes homogeneous. Once uniform, the mixture is transferred to a container and placed on a magnetic stirrer. Then, 20 mL of the chitosan solution is added and stirred at 1200 rpm for 15 minutes. Subsequently, a 0.1% NaTPP solution is slowly incorporated while stirring is maintained for an additional hour. Finally, the 0.1% NaTPP solution is added incrementally, ensuring continuous agitation for one hour (Yan et al., 2020). After completing the mixing process, we analyze the attributes of the nanopolyherbal formulation to determine its quality and stability.

2.2.3 Characteristic Test of Nanopolyherbal

Nanoparticle characterization was performed utilizing a Malvern Zetasizer Nano ZS90 (Malvern Panalytical, UK), that employs dynamic light scattering (DLS) by non-invasive backscatter (NIBS) checking. To ensure accurate measurements, the nanosuspension was diluted 100-fold-100 μL of sample in 10 mL of ultrapure water (resistivity 18.2 MΩ·cm) -to achieve optimal photon count rates inside of the instrument's sensitivity range. The hydrodynamic diameter and polydispersity index (PDI) were measured in triplicate at a backscatter angle of 173°, utilizing disposable polystyrene cuvettes (Sarstedt, Germany) degree was precisely maintained at 25.0 ± 0.1°C by a Peltier-controlled thermal block. Dynamic light scattering (DLS) gived intensity-weighted size distributions, by the PDI serving as a quantitative indicator of sample uniformity; values below 0.3 reveald a monodisperse system. Zeta capability was measured utilizing a Malvern Zetasizer (model 1203893) to determine the electrical charge of the nanoencapsulation system, a key indicator of dispersion stability. Measurements were based on electrophoretic mobility, by 100 μL of nanosuspension diluted in 10 mL of distilled water and placed in an electrode-equipped cuvette (Patel and Agrawal, 2011).

Drug-excipient interactions and crystallinity inside of the formulation were investigated utilizing Fourier Transform Infrared Spectroscopy (FT-IR) by a Shimadzu FT-IR 8300 spectrophotometer and Differential Scanning Calorimetry (DSC) by a Shimadzu DSC-60, over a wavelength range of 4000–400 cm^{-1}. Particle morphology was tested by Transmission Electron Microscopy (TEM). For this, a diluted nanosuspension sample was placed onto a TEM grid and allowed to rest for one minute to ensure uniform particle distribution. Excess liquid was then omitd by filter paper, and the grid was dried before detailed size and morphology analysis under the TEM.

2.2.4 In Vitro Antioxidant Test

To measure Nrf2 and GST stages by flow cytometry, PBMCs were initially washed by PBS containing 10% endotoxin-free FBS. The cells were then fixed by 1% paraformaldehyde (PAF) solution for 20 minutes at room degree to preserve protein structure and integrity. Following fixation, cell surface markers were labeled utilizing specific dye-conjugated antibodies: CD3-FITC for T cells, CD14-FITC for monocytes, and CD19-FITC for B cells. After isolation, PBMC suspensions underwent three consecutive washes (5 minutes each, 300 × g, at 4°C) utilizing ice-cold phosphate-buffered saline (PBS; pH 7.4) supplemented by 1 μM ethylenediaminetetraacetic acid (EDTA) to reduce cell aggregation. The purified PBMCs were then cultured for 48 hours in RPMI 1640 medium (Gibco, Thermo Fisher Scientific) supplemented by 10% (v/v) heat-inactivated fetal bovine serum (FBS; qualified, US origin), 2 mM L-glutamine, and 0.1% (v/v) gentamicin sulfate (50 mg/mL). Cultures were maintained under controlled conditions at 37°C, 5% CO_2, and 95% relative humidity utilizing a HERAcell 150i CO_2 incubator (Thermo Scientific). To ensure consistency, fresh PBMC aliquots were prepared for each independent assay (n = 3 biological replicates), by all measurements performed in technical duplicates (Raulf, 2020). To measure Nrf2 and GST stages by flow cytometry, PBMCs were initially washed by PBS containing 10% endotoxin-free FBS. Cells were fixed by 1% paraformaldehyde (PAF) for 20 minutes at room degree to preserve protein structure and integrity. After fixation, cell surface markers were labeled by specific dye-conjugated antibodies: CD3-FITC for T cells, CD14-FITC for monocytes, and CD19-FITC for B cells. Staining was performed for 45 minutes at 4°C in the dark, followed by two washes by PBS containing FBS. Intracellular proteins were then stained-Nrf2 by anti-Nrf2 antibodies and GST by anti-GST antibodies conjugated to the PE fluorochrome. This staining step was figured out for 60 minutes at room degree, protected from light. To identify cell nuclei and exclude dead cells, 7-AAD DNA dye was added at a concentration of 0.25 μg per 10^6 cells and incubated for 10 minutes (Guan et al., 2023; Jiang et al., 2010). After finishing all the labeling steps, the cells are washed and prepared for analysis utilizing a flow cytometer by CellQuest Pro™ software.

2.2.5 In Vitro Glycation Inhibition Test

Advanced glycation end-product (AGEs) in peripheral blood mononuclear cells (PBMCs) were quantified utilizing an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's standardized protocol. Initially, 50 μL of fixed PBMCs were added to wells containing AGE conjugate and incubated at room degree for 10 minutes. Following this, 50 μL of anti-AGE antibody solution was added to each well and incubated for one hour at room degree. Following this step, the wells were carefully washed three times by 250 μL of wash buffer to omit unbound antibodies. After the final wash, the wells were emptied and gently tapped on tissue to omit excess buffer. Next, 100 μL of the HRP-secondary antibody conju-

gate was added to each well and incubated at room degree for 2 to 20 minutes. Careful monitoring of this step is essential, as rapid color enhancement requires promptly stopping the reaction to prevent sample saturation. The reaction was halted by adding 100 μL of stop solution to each well following secondary antibody incubation.Once the reaction has been stopped, the absorbance of each well is immediately measured at 450 nm utilizing an ELISA reader to quantify the AGE content in the sample. (Perrone et al., 2020; Xu et al., 2010).

3. RESULTS AND DISCUSSION

3.1 Characteristic Nanoencapsulation Polyherbal

Particle Size Analysis (PSA) showed that the particles in the polyherbal nanosuspension have an average size of 186 ± 46.81 nm. The components of the formulation-particularly Tween 80, chitosan, and NaTPP-significantly influence the final particle size. Nanoparticles are generally expected to enhance the bioavailability of active compounds. Furthermore, smaller particle diameters can affect the absorption of the active ingredient, as well as the rate and extent of its release from the polymeric nanoparticle system (Bilia et al., 2014). The polydispersity index (PDI) was recorded at 0.434 ± 0.04, reflecting the distribution of particle sizes and indicating the degree of homogeneity inside of the sample. A high PDI value (≥ 0.5) suggests considerable variation in particle size, whereas a value closer to zero denotes a more uniform size distribution and greater homogeneity (Danaei et al., 2018). The zeta capability measurement yielded a value of -29.69 ± 1.54 mV. This characterization was conducted to analyze the stability of the nanopolymeric formulation containing polyherbal extracts. A higher zeta capability, whether positive or negative, reflects stronger repulsive forces among particles, that help prevent aggregation. In pharmaceutical and colloidal formulations, a significant zeta capability-regardless of polarity-is beneficial, as it reveals effective electrostatic stabilization and enhances the system's resistance to particle clumping (Németh et al., 2022). These characterization outcomes are summarized in Table 1, confirming the nanopolymeric formulation's stability and effectiveness for delivering polyherbal extracts.

The outcomes of the stability test for the nanopolyherbal formulation-comprising particle size, polydispersity index (PDI), and zeta capability-are presented in Table 2. The stability tests showed no changes in color, by sediment remaining consistent throughout the storage period. Particle size gradually increased from day 1 to day 60, likely due to agglomerate construction over time. Nevertheless, the polydispersity index (PDI) remained inside of an acceptable range, and although the zeta capability exhibited a slight decline, it remained sufficiently high to maintain strong repulsive forces among particles, that are essential for sustaining the formulation's stability. The outcomes match what Fan et al. (2012) found, showing that when stored at low degrees among 10°C and 25°C, the size of the nanokitosan particles stayed small. Additionally, the research by López-León et al. (2005) showed that at -10°C, the chitosan nanoparticle solution was unstable, but at 5°C and 25°C, the size of the particles grew significantly on the 7th and 15th days.

Transmission Electron Microscopy (TEM) analysis, shown in Figure 1, reveals that the particles in the nanosuspension are spherical and exhibit a small, uniform size distribution. by an approximate particle size of 50 nm, they fall inside of the ideal nanometer range for nanosuspensions, supporting enhanced solubility, controlled release of active compounds, and increased bioavailability. The consistent particle shape also reveals that the surfactants effectively stabilize the particles and prevent aggregation, capabilityly improving the overall distribution of active ingredients throughout the nanosuspension.

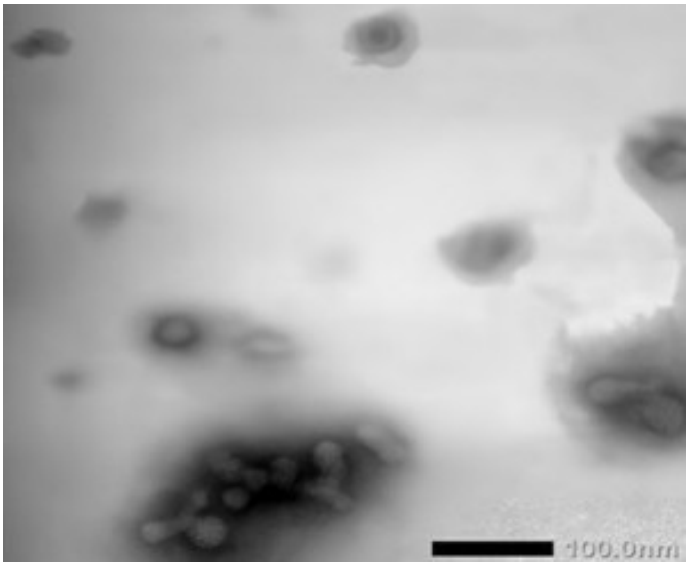

Figure 1. Morphological Outcomes of Nanopolyherbal Utilizing TEM

Fourier Transform Infrared Spectroscopy (FTIR) analysis of *Apium graveolens, Centella asiatica,* and *Orthosiphon stamineus* extracts, as well as their combination and the polyherbal nanosuspension, revealed a prominent peak among 1650 and 1630 cm^{-1}, indicating the presence of aromatic C=C groups in these substances (Figure 2). The findings correspond by the research by Nomi et al. (2024) and Krysa et al. (2022), that performanced that absorptions inside of the ranges of 1600–1450 cm^{-1} and 1700–1660 cm^{-1} are indicative of C=C bonds in triterpenoids or carbonyl groups (C=O). The absorption at 1400–1500 cm^{-1} reveals O−H bending, characteristic of phenolic compounds, as shown by (Masfria et al., 2023). The peak at 1272.5 cm^{-1} reveals C−O stretching Sugunabai et al. (2015) while the absorption range of 1200–1000 cm^{-1} denotes the presence of flavonoids and triterpenoids, acknowledged for their antioxidant and anti-inflammatory attributes (Auliya Putri et al., 2023). The diminished absorption at 1405 cm^{-1} and 1274 cm^{-1} in the spectrum of the polyherbal nanosuspension suggests a change in particle size, that impacts the intensity of the infrared spectrum (de Silva et al., 2023). The variations observed in the spectral patterns among the nanopolyherbal

Table 1. Nanopolyherbal Evaluation

Nanopolyherbal	Particle Size (nm)	Polydispersity Index	Zeta Capability (mV)
Replication 1	139.19	0.58	-31.23
Replication 2	186.00	0.62	-29.69
Replication 3	232.81	0.66	-28.15
Mean ± SD	186 ± 46.81	0.62 ± 0.04	-29.69 ± 1.54

Table 2. Stability of Nanopolyherbal Particles at Room Degree

Parameters	Day-1	Day-15	Day-30	Day-60
Color change	greenish clear	Greenish clear	Greenish clear	Greenish clear
Sediment	yes	yes	yes	yes
Particle Size (nm)	186 ± 46.81	187.33 ± 1.15	188.67 ± 1.53	193.67 ± 4.04
Polydispersity Index	0.62 ± 0.04	0.63 ± 0.03	0.64 ± 0.03	0.69 ± 0.01
Zeta Capability (mV)	-29.69 ± 1.54	-29.37 ± 0.40	-28.94 ± 0.127	-28.12 ± 0.37

formulation and the individual extracts suggest interactions among the active compounds and the polymer matrix, that could enhance formulation stability and prolong the release of the active ingredients (Sinha et al., 2021). These outcomes are consistent by the research conducted by Udvardi et al. (2017), that performanced that particle size affects the FTIR spectrum.

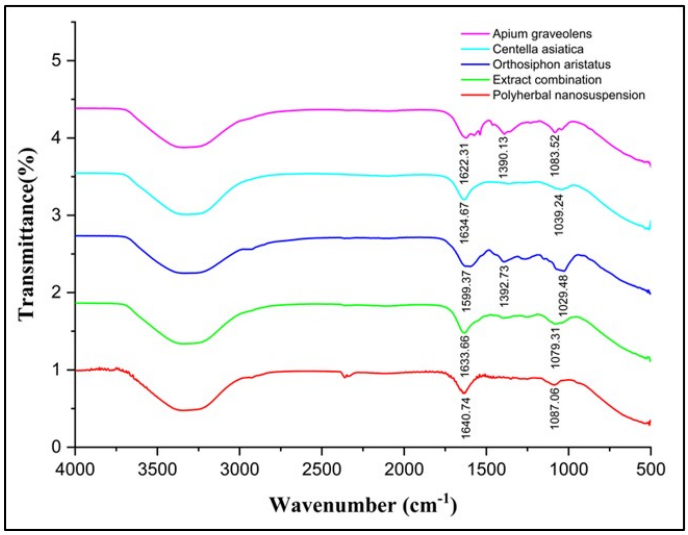

Figure 2. FT-IR Spectroscopy Outcomes

3.2 Antioxidant Effect by Expression Nrf2 and GST

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a key transcriptional regulator of the body's natural antioxidant defense system. It promotes the expression of cytoprotective genes encoding phase II detoxification enzymes and molecules involved in maintaining redox balance. Upon activation, Nrf2 translocates to the nucleus, where it binds to antioxidant response elements (AREs) and initiates the transcription of various protective proteins. These proteins work together to reduce reactive oxygen species (ROS) and maintain cellular redox homeostasis (Hassanein et al., 2024). Nrf2 plays a vital framework in maintaining cellular health by enhancing NADPH itemion, neutralizing toxic substances, and preserving DNA integrity, thereby helping to prevent mutations and degenerative condition. Consequently, Nrf2 has arisen as a indicative of success therapeutic target for a range of conditions associated by oxidative stress, comprising diabetes, cardiovascular condition, and neurodegenerative disorders (David et al., 2017; Satta et al., 2017).

Oxidative stress is a complex pathological condition outcoming from an imbalance in the body's redox homeostasis. It occurs when the itemion of reactive oxygen species (ROS) and reactive nitrogen species (RNS) exceeds the load of the body's natural antioxidant defenses. This imbalance initiates a cascade of harmful molecular events, comprising disruption of redox-sensitive signaling pathways and oxidative damage to critical cellular molecules (David et al., 2017). Consequently, this imbalance induces deleterious modifications at the cellular stage, as reactive oxygen species (ROS)-encompassing superoxide radicals ($O^{\bullet -}$), hydrogen peroxide (H_2O_2), nitric oxide (NO), and peroxynitrite ($ONOO^-$)-exhibit pronounced chemical reactivity, outcoming in oxidative damage to biomolecules and cellular structures (García-Sánchez et al., 2020). Reactive oxygen species (ROS) are primarily generated inside of cells through mitochondrial metabolic activities, whereas reactive nitrogen species (RNS) originate from nitric oxide (NO), a signaling molecule enzymatically produced by nitric oxide synthase (NOS). Under normal physiological conditions, nitric oxide acts as an antioxidant at low stages. However, at higher concentrations, it can paradoxically promote oxidative stress, exacerbating the progression of various condition. Therefore, enhancing Nrf2-mediated signaling pathways represents an important therapeutic approach to reduce oxidative damage and maintain cellular redox balance (Wu et al., 2022).

Figure 3 demonstrates that treatment by the polymer-based nanopolyherbal formulation outcomes in a statistically significant increase in Nrf2 expression compared to the untreated

control group, indicating enhanced activation of the antioxidant response pathway. The transcriptional activation of nuclear factor erythroid 2-related factor 2 (Nrf2) is a key regulatory process that controls the itemion of endogenous antioxidant enzymes, that collectively give essential protection to cells against oxidative damage. The increased expression of Nrf2-regulated genes suggests that the tested formulation effectively enhances cellular defense mechanisms against reactive oxygen species (ROS). This enhancement may help mitigate the harmful effects of oxidative stress, that is implicated in the enhancement of various degenerative condition.

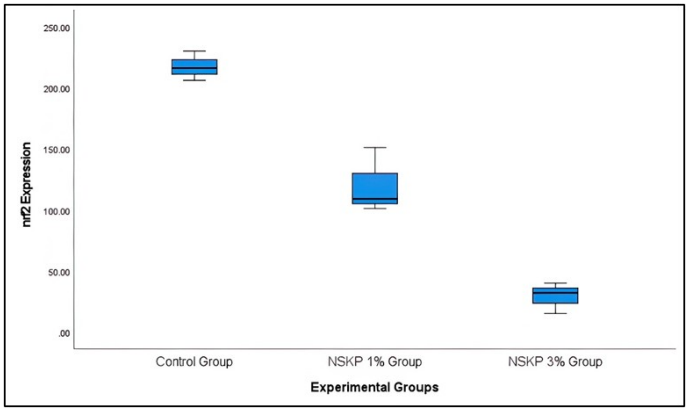

Figure 3. Nrf2 Expression in Experimental Groups

Glutathione S-transferase (GST), a key element of the cellular detoxification system, catalyzes the conjugation of electrophilic substrates by decreased glutathione (GSH). This process not only neutralizes and promotes the removal of harmful endogenous and exogenous compounds but also helps alleviate oxidative stress by maintaining redox balance (Singhal et al., 2016). Enzyme activity at the transcriptional stage is regulated by the redox-sensitive transcription factor Nrf2, that undergoes conconstructional changes upon oxidative modification, leading to its release from the inhibitory Keap1 complex. Once activated, Nrf2 translocates into the nucleus, where it forms a heterodimer by small Maf proteins and binds to antioxidant response elements (AREs) inside of specific regulatory regions of the genome. This interaction activates the transcription of numerous cytoprotective genes, particularly those encoding phase II xenobiotic-metabolizing enzymes such as glutathione S-transferase (GST), thereby coordinating a broad cellular response to oxidative stress (Feng et al., 2023). Glutathione S-transferase (GST) functions as a key molecular guardian in cellular defense by catalyzing the nucleophilic conjugation of decreased glutathione (GSH) to a broad range of electrophilic compounds, comprising reactive oxygen species (ROS) and toxic lipid peroxidation items such as 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA). This enzymatic process plays a critical framework in detoxification by preventing the concentration of oxidative damage markers. These markers, through covalent bonding by cellular proteins and structural alterations of key macromolecules, can initiate pathological processes that lead to cellular dysfunction and disruption of organ homeostasis if left unchecked.

Beyond its well-known framework in xenobiotic detoxification, glutathione S-transferase (GST) also functions as a regulator of key cellular signaling pathways, particularly those involved in inflammation and programmed cell death. This regulatory effect arises from GST's ability to modulate the phosphorylation and nuclear translocation of mitogen-activated protein kinase (MAPK) family members, particularly c-Jun N-terminal kinase (JNK), while simultaneously disrupting the activation process of the redox-sensitive transcription factor nuclear factor kappa B (NF-kB) (Doney et al., 2005; Al Hroob et al., 2019). Activation of the Nrf2-Keap1-ARE signaling pathway is crucial for regulating immune responses and enabling cellular adaptation to oxidative stress. This pathway orchestrates the coordinated expression of cytoprotective genes. Upon release from its cytoplasmic inhibitor Keap1, nuclear factor erythroid 2-related factor 2 (Nrf2) translocates into the nucleus and binds to antioxidant response elements (AREs) inside of target genomic regions. This interaction initiates the transcription of phase II detoxification enzymes, comprising glutathione S-transferase (GST). Collectively, this molecular network gives a robust defense against oxidative stress and low-grade inflammation. As a outcome, it helps to reduce the molecular mechanisms driving complex polygenic disorders, especially those related to the microvascular complications of chronic hyperglycemia in diabetes mellitus, as well as the endothelial dysfunction that promotes atherogenesis in cardiovascular diseas (Pahwa et al., 2017).

The quantitative analysis of glutathione S-transferase (GST) expression, as shown in the accompanying figure, reveals a dose-dependent increase following treatment by nanopolyherbal formulations. Figure 4 shows that treatment by the polymer-based nanopolyherbal formulation outcomes in a significant increase in GST expression compared to the untreated control group, indicating enhanced activation of the antioxidant response pathway. Notably, both 1% and 3% concentrations produced statistically significant elevations in GST expression (p <0.05) compared to controls, as performanced by Western blot analysis. This marked increase in GST catalytic activity reflects a substantial enhancement of the body's natural detoxification system. This boost may give cardioprotective benefits through two interconnected mechanisms: (1) effectively neutralizing reactive oxygen species (ROS) to reduce oxidative damage to macromolecules, and (2) modulating key pro-inflammatory signaling molecules, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), thereby helping to suppress chronic low-grade inflammation. These experimental findings highlight the capability clinical significance of nanopolyherbal formulations in cardiology. Specifically, they demonstrate the ability of these formulations to mitigate oxidative stress–related cellular damage and inflammation-driven pathological remodeling, both of that play critical frameworks in the enhancement and progression of cardiovascular dysfunc-

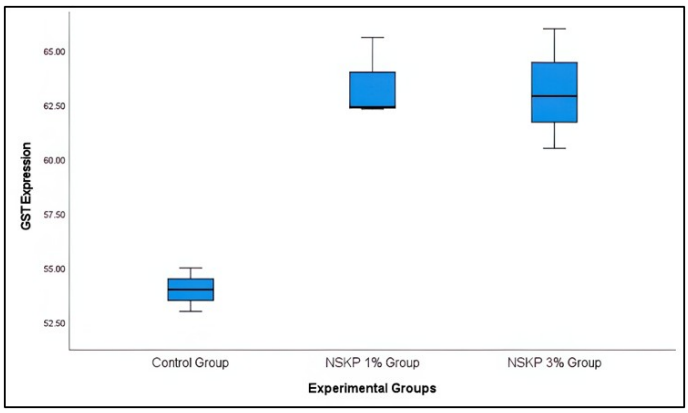

Figure 4. Gst Expression in Experimental Groups

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3.3 Antiglicatyon Test

Advanced glycation end product (AGEs) are a diverse group of biologically active compounds formed through non-enzymatic glycation reactions. In this process, decreasing sugars such as glucose or their derivatives spontaneously react by amino groups in proteins, lipids, or nucleic acids, outcoming in chemical modifications (Perrone et al., 2020). The endogenous itemion of AGEs is significantly increased under hyperglycemic conditions. While this process occurs naturally during normal aging in individuals by typical blood glucose stages, it is markedly amplified in pathological states such as diabetes mellitus (DM). In DM, persistent hyperglycemia accelerates AGE concentration, that plays a crucial framework in the progression of cardiovascular complications associated by the disease (Bansal et al., 2023). Beyond cautilizing direct oxidative damage, free radicals play a pivotal framework in accelerating the endogenous construction of AGEs by activating redox-sensitive signaling pathways. This process is primarily driven by elevated stages of reactive oxygen species (ROS), that initiate key signaling cascades, comprising those mediated by c-Jun N-terminal kinase (JNK) and oxidative bursts involving NADPH oxidase. These molecular disturbances impair mitochondrial energy itemion, thereby exacerbating oxidative stress and establishing a self-perpetuating cycle of cellular damage that further promotes AGE concentration (Rungratanawanich et al., 2021).

Recent research suggests that AGEs could serve as valuable clinical biomarkers for the early checking and monitoring of cardiovascular complications associated by diabetes mellitus (DM). The pathophysiological effects of AGEs occur through two main mechanisms: first, by cautilizing structural stiffening in vascular and myocardial tissues through the construction of irreversible cross-links by extracellular matrix proteins; and second, by promoting chronic low-grade inflammation via binding to the receptor for advanced glycation end product (RAGE), that activates downstream pro-inflammatory signaling pathways, comprising NF-kB and MAPK (Chen et al., 2023a). AGEs, formed through non-enzymatic glycation reactions, tend to accumulate more extensively in individuals by hyperglycemia, impaired kidney function, or prolonged exposure to tobacco smoke. These compounds have been detected in cardiac tissue, blood vessels, and atherosclerotic plaques, highlighting their framework in cardiovascular pathology. Consequently, AGEs are gaining attention as capability therapeutic targets to slow the progression of cardiovascular disease, particularly in recipients by coexisting diabetes mellitus (CVD-DM) (Siam et al., 2024). The binding of AGEs to their specific receptor (RAGE) triggers a cascade of intracellular signaling events, comprising activation of pathways such as MAPK, ERK, p21ras, p38, and JAK-STAT. This signaling ultimately enhances the itemion of reactive oxygen species (ROS) via NADPH oxidase, playing a central framework in promoting oxidative stress. Activation of the pro-inflammatory NF-kB signaling pathway leads to increased itemion of key inflammatory mediators, comprising tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), and cohesion molecules such as vascular cell cohesion molecule-1 (VCAM-1) and intercellular cohesion molecule-1 (ICAM-1). This response exacerbates systemic inflammation and disrupts vascular homeostasis. Consequently, the AGE-RAGE signaling axis plays a pivotal framework in the pathogenesis of atherosclerosis, ischemic cardiomyopathy, congestive heart failure, and cerebrovascular events, by significant implications for individuals affected by diabetes mellitus (Perrone et al., 2020).

Statistical analysis revealed that individuals treated by the nanopolyherbal formulation exhibited a significant reduction in AGE stages compared to untreated control groups. Interestingly, dose-response comparisons among the 1% and 3% treatment groups showed that the lower concentration (1%) was associated by a more pronounced decrease in AGE concentration. These findings suggest that the nanopolyherbal formulation exerts a dose-dependent inhibitory effect on AGE biosynthesis, by peak efficacy observed at specific concentration thresholds. The quantitative variability and distribution of these outcomes are illustrated in the accompanying box plot. Figure 5 shows that treatment by the polymer-based nanopolyherbal formulation induces a statistically significant reduction in AGEs stages compared to the untreated control group, indicating enhanced activation of antiglycation defense mechanisms.

The outcomes of our research agree by those of Zhou et al. (2019), these findings give evidence that the phytochemical apigenin effectively mitigates the deleterious effects of oxidative stress and inflammatory responses induced by the construction of AGEs inside of human umbilical vein endothelial cells (HUVECs). Researchers Liu et al. (2021) also found that the chemical sinensetin can stop α-glucosidase by working and stop the process of non-enzymatic glycation by happening. This intrinsic mechanism promotes glycemic regulation by directly modulating circulating plasma glucose stages while simultaneously inhibiting the non-enzymatic glycation pathways responsible for generating AGEs in individuals diagnosed by established type 2 diabetes mellitus (T2DM). Legiawati et al.

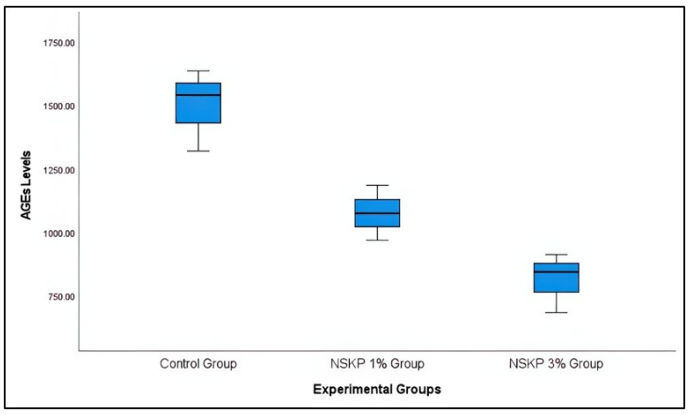

Figure 5. AGEs-ELISA Measurements in Experimental Groups

(2023) outcomes also support the idea that the active compounds in *Centella asiatica* (CA) can slow down the aging process. The bioactive constituents include asiaticoside, madasiatic acid, and madecassic acid, each of that, according to empirical studies, exhibits significant inhibitory activity against the biosynthesis of AGEs and their binding affinity to the RAGE. This bifunctional mode of action effectively downregulates proinflammatory signaling pathways while concurrently alleviating oxidative stress through complex molecular interactions, as performanced by advanced computational molecular dynamics simulations. Notably, the observed synergistic interplay among the diverse phytochemical components gives strong evidence supporting caffeic acid (CA) as a indicative of success therapeutic candidate for preventive strategies against pathological conditions driven by AGEs.

4. CONCLUSIONS

The investigation demonstrates that the polyherbal nanopolymeric formulation exhibits small particle sizes, averaging approximately 186 nm, by a uniform size distribution reveald by a polydispersity index (PDI) of 0.434, and high colloidal stability, as reflected by a zeta capability of –29.69 mV. Fourier-transform infrared (FTIR) spectroscopy further reveals molecular interactions among the bioactive compounds and the polymer matrix, collectively enhancing the formulation's physicochemical stability. Furthermore, the study demonstrates that this composite system effectively elevates the expression stages of Nrf2 and GST, both of that play critical frameworks in cellular defense against oxidative stress-induced damage. Moreover, the formulation demonstrates significant efficacy in inhibiting the construction of AGEs, particularly at a 1% concentration, thereby offering capability to mitigate complications commonly associated by diabetes and cardiovascular condition. These findings suggest that the nanosuspension represents a indicative of success strategy for enhancing drug delivery in the management of degenerative disorders.

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