RESEARCH PAPER

In vitro anti-arthritic and antiglycation potential of a combination of silver nanoparticles and *Moringa oleifera* leaves extract

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ABSTRACT

Objective(s): The present work aimed to investigate the efficacy of glycation inhibitors and the anti-arthritic potential of *Moringa oleifera* mediated silver nanoparticles (MO-AgNPs).

Materials and Methods: Aqueous leaf extract of *Moringa oleifera* is used as a reducing and stabilizing agent for the synthesis of silver nanoparticles. UV–vis spectrophotometer analysis, high resolution-transmission electron microscopy (HR-TEM), and Fourier-transform infrared spectroscopy were performed to characterize the synthesized AgNPs. The antioxidant potential of plant leaf extract and MO-AgNPs was studied by a free radical (DPPH) scavenging assay. *In-vitro* BSA-glucose glycation assay and a protein denaturation assay were carried out to determine protein anti-glycation activity and anti-arthritic potential respectively for MO-AgNPs and compared with MO-leaves extract.

Results: Aqueous extract of *Moringa oleifera* shows total phenolic content (163.5 ± 4.73 mg of GAE/g) and flavonoid content (56.8 ± 7.23 mg quercetin equivalents/g). Moringa oleifera leaves mediated AgNPs were confirmed by surface plasmon resonance at 420–440 nm in the UV-Visible spectra. HR-TEM analysis indicates the crystalline nature of the MO-AgNPs with spherical and spheroid shapes. FTIR spectra suggested the presence of phenolic, flavonoid, and alkaloids in plant extract responsible for the reduction and capping material of AgNPs. DPPH radical scavenging activity of MO-AgNPs shows significantly (P>05) higher with its IC_{50} value (62 µg/ml) than MO-leaf extract (95 µg/ml). MO-AgNPs exhibited (45%) higher anti-arthritic activity compared to moringa leaves extract but were similarly found with the standard diclofenac sodium drug. MO-AgNPs exhibited a strong inhibitory effect on AGEs formation that was predicated on the reduced levels of fructosamine and fluorescence intensity.

Conclusion: This work demonstrated that moringa leaves mediated AgNPs prevent protein glycation and arthritic activity invitro. Thus, this finding depicts the anti-arthritic and antiglycation potential of MO-AgNPs suggesting that it can be a promising Nano-carrier to prevent the progression of arthritis and the pathogenesis of AGEs-related diseases.

Keywords: *Anti-arthritis, Antiglycation, Moringa oleifera, Silver nanoparticles*

How to cite this article
Verma D, Macwan D, Mangrola AV, Solanki SG, Bariya HS, Patel HV. In vitro anti-arthritic and antiglycation potential of a combination of silver nanoparticles and Moringa oleifera leaves extract. Nanomed J. 2022; 9(4): 334-344. DOI: 10.22038/NMJ.2022.67265.1709

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease characterized clinically by joint edema, lymphocytic and synovial lining cell intrusion, vascular propagation, and obliteration of cartilage and bone with a global prevalence of 0.3%–1% according to the World Health Organization. According to the Arthritis Foundation, approximately two-thirds of the population suffers from arthritis, and the census predicts that this figure will rise above 40% by 2030 (1). The signs of RA include inflexibility or rigidity of the affected joints, exhaustion, fever, weight loss, joints that are tender, swollen, and warm, and nodules under the skin. Inflammatory cells, proteolytic enzymes, and free radicals derived

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Note. This manuscript was submitted on July 10, 2022; approved on September 24, 2022

from oxygen species abundantly populate the affected joints. The free radicals are responsible for the breakdown of cartilage and bone in this scenario (2). Oxidative stress stimulates protein glycation and has been implicated in the development of arthritis. Chronic inflammation might consequence in the creation of advanced glycation end products (AGEs). AGEs are formed and can accrue during chronic inflammation, as might be present in patients with rheumatoid arthritis (RA). In the previous study, glycated protein and amino acids were accumulated in the synovial fluid and plasma of arthritic patients (3). Glycation of protein present in cartilage tissue leads to a dysfunctional proteoglycan–collagen network and increased matrix degradation. (4). The persistence of RA and AGEs is increased due to prolonged exposure to oxidative stress. The ultimate goals of treatment for RA are to stop or at least decrease joint inflammation and pain and to prevent joint destruction and deformity (5). The existing treatment options for RA do not provide complete diminution and are often associated with serious systemic side effects, thereby convincing patients to opt for alternative medicine. So, for redefining the treatment with amplified potency and nominal toxicity, recent technologies have been united with natural products. One such alternative treatment is Phyto-nanomedicine, a promising field that provides targeted therapy with the help of phytoconstituents and nanocarriers. Nanocarriers are drug transport modules of submicron size (6).

It has been established that the nanocarriers can carry the drug to the target site and accumulate specifically owing to their passive targeting mechanisms, by continuously supplying the encapsulated agent to the site (7). Among the different nanocarriers, metal nanoparticles are often used due to their known antimicrobial and anti-inflammatory effects (8). Among the metal nanoparticles, silver nanoparticles take advantage of their high-surface-to-volume ratio to increase the contact area, resulting in a higher amount of silver ions to perform their activities (9). Nanoparticles delivered drug components to the targeted site through natural transport mechanism. Medicinal plants and herbs are a source of an unlimited variety of phytochemicals that continuously proved to be effective in preventing, treating, or ameliorating different health conditions, including glycation reversing ability (10). *Moringa oleifera* is grown in many countries including India, Bangladesh, and Pakistan, and is one of the promising plants because it contains several compounds that have anti-oxidants and anti-inflammatory and immunomodulatory effects (11, 12). *Moringa oleifera* is widely used in traditional medicine against the denaturation of proteins, which shows that it could be used as an antiarthritic agent (13). Aqueous extract of moringa is widely used for the therapy of arthritis as it can reduce the level of rheumatoid factor, TNF-alpha, and IL-1 in arthritic experimental animals (14). This fact meets a critical need for *in-vitro* evaluation of plant-mediated nanocarriers by using laboratory methods. We hypothesized that the active phytochemical constituent of *Moringa oleifera* capped on nanoparticles as carriers to the site of joint inflammation and prevented further progression of arthritis. Therefore, the present study was carried out to study the anti-oxidant, anti-arthritic, and antiglycation activities of *Moringa oleifera* medicated silver-nanocarriers.

MATERIALS AND MATHODS

Collection and extraction of samples

Healthy, mature, and fresh leaves of *Moringa oleifera* were collected from the campus of Agriculture University, Anand, Gujarat, and then separated from the stalks, washed with distilled water, and shade dried for 8 to 12 days with regular shifting. The dried sample was ground into fine powder form and subjected to extraction using a Soxhlet extractor with distilled water as the solvent and stored at 4ºC until use.

Phytochemical analysis of the extract

The crude aqueous extract of *M. oliefera* was then subjected to qualitative phytochemical screening to detect the presence of various chemical constituents such as phenols, tannins, steroids, carbohydrates, flavonoids, and saponins, terpenoids, and alkaloids (15).

Determination of total phenolic content (TPC)

The total phenolic content of the crude extract was determined using the FCR (Folin-Ciocalter reagent) method with gallic acid as a reference (16). In this test, about 0.5 ml of the extract was taken and 2.0 ml of the FCR reagent was added and incubated for 2 minutes at room temperature, which was then neutralized by the addition of 2 ml of 20% (w/v) sodium carbonate. This combination

was allowed to sit at room temperature for 60 minutes in the dark. Absorption was taken at 650 nm in a spectrophotometer. The total phenolic content was estimated using the gallic acid calibration curve, and the results were represented in mg of garlic acid equivalent per g of dry weight.

Determination of total flavonoid content (TFC)

Using an aluminum chloride colorimetric method, the flavonoid content of the crude extract was determined (17). The sample solution (0.2 ml) was combined with 2.5 ml of 10% aluminum chloride solution, 2.5 ml of sodium acetate (1.0 M), and 4.9 ml of distilled water. This mixture was incubated at room temperature for 15 minutes in the dark. After incubation, the absorbance was measured at 415 nm on a UV visible spectrophotometer against a blank. Quercetin was used as a standard (0-80 mg/ml). The amount of total flavonoid content was expressed in mg of quercetin equivalent/g of dry weight.

Bio-synthesis of MO-AgNPs

The synthesis of MO-AgNPs was performed according to the methodology by Elbagory et al. (18) with slight modifications. 10 ml of aqueous leaves extract was added dropwise to 100 ml of 1.0 mM aqueous solution of silver nitrate by continuing stirring on a magnetic stirrer at a controlled temperature (60 ˚C) for 2 hr in the dark to minimize the photo-activation of silver nitrate. The reduction of Ag+ to Ag0 is confirmed by the color change from colorless to a colloidal red-brown color. This mixture was incubated at 25̊C and then purified by centrifugation at 10,000 rpm for 10 min. The sediment pellet of nanoparticles was lyophilized and stored for further characterization.

Characterization of the nanoparticles

The UV-visible spectroscopy of the synthesized nanocarriers was performed using a double beam UV-visible spectrophotometer (Systronic, 2022) by estimating the plasma resonance and assessing the collective oscillations of conduction band electrons in response to electromagnetic waves (19). The blank solution was distilled water. Silver nanoparticles produce a specific absorbance peak between 400 and 450 nm due to the excitation mode of the surface plasmon, which extends depending on the size of the nanoparticles (20). The reduction of silver ions and the formation of silver nanoparticles were formed within an hour of the reaction. The absorption of light at a specific wavelength is characteristic of the type of chemical bond seen in the annotated spectrum. 50 µl of synthesized nanoparticles were directly subjected to translucent sample discs and were loaded into an FTIR spectroscopy with a scan range from 400 to 4000 cm-1 having a resolution of 4 cm-1 (21). The morphology and size of silver nanocarriers were confirmed by HR-TEM. Nanoparticles were coated and developed on a copper grid of 200 mesh size and allowed to dry before observation at a voltage of 200kV. The selected area electron diffraction (SAED) pattern was also documented.

DPPH free radical scavenging assay

The free radical scavenging activity of aqueous extract and MO-AgNPs was measured using the stable radical DPPH (2, 2-diphenyl-2-picryl-hydroxyl) (22). 1.0 ml of 0.1 mM DPPH in methanol was added to an equal volume of sample with different concentrations. The reaction mixture was shaken well and incubated for 30 min in the dark. Absorbance was measured at 517 nm against a blank (methanol). Ascorbic acid was taken as the standard. The lower the absorbance of the reaction mixture, the greater the percentage of scavenging activity. IC_{50} values denote the concentration of the sample, which is required to scavenge 50% of DPPH free radicals. The percentage of inhibition or scavenging of free radicals was determined by the following formula: % Inhibition = [(Absorbance of Control-absorbance of the sample) /Absorbance of control] x 100.

Anti-arthritic assay

The anti-arthritic assay was performed by using the method described by Amoolya et al. (23). 0.05 ml of standard diclofenac sodium, plant extract, and MO-AgNPs with various concentrations were added to 0.45 ml of bovine serum albumin to bring the final volume to 0.5 ml. All the samples were kept in the incubator for 20 minutes at 37˚C, and later the temperature was raised to keep the sample at 72˚C for 5 minutes. After cooling, 2.5 ml of phosphate buffer was added to all the samples. The absorbance was measured at a wavelength of 660 nm using a visible spectrophotometer. The control represents complete protein denaturation. The result of the sample was compared with the standard value of diclofenac sodium. The inhibition percentage was determined using the following formula: % inhibition of denaturation = 100 × (1−A2/

A1), where A1 = absorption of the control sample, and A2 = absorption of the test sample.

In vitro anti-glycation assays using the bovine serum albumin (BSA-glucose) model

The evaluation of the inhibition of the protein glycation was performed according to the method described by Matsuura et al. (24) with some modifications. The nanoparticles, crude aqueous extract, and aminoguanidine (100, 200, 500 µg/ml) dissolved in distilled water containing 0.02% sodium azide with BSA (10 mg/ml) and 500 mM of phosphate buffer for a final volume of 5 ml at pH 7.4 were incubated at 37°C for four weeks. Aminoguanidine was used as a positive inhibitor. After four weeks of incubation, the fructosamine level was determined using a nitro blue tetrazolium (NBT) assay (25). 100 µl of glycated samples were incubated with 900 µL of 0.5 mM NBT in 100 mM carbonate buffer (pH 10.4) at 37°C and absorbance was recorded at 530 nm compared with the standard 1-deoxy-1-morpholino-fructose (1-DMF) curve. The fluorescent intensity of glycated protein was measured using a fluorescent spectrometer with an excitation wavelength of 370 nm and an emission wavelength of 440 nm. The ability of nanoparticles to inhibit the formation of AGEs was calculated by the equation (FI: fluorescence intensity): Inhibition [%] = {1−[(FI of the sample)/(FI of blank)]} × 100.

Statistical analysis

The results were analyzed using SPSS software (SPSS 12.0 for Windows) and presented as the mean ± SD of three independent measurements. The level of significance was set at $P<0.05$.

RESULTS

Phytochemical screening

The result of qualitative analysis to detect the presence of various bioactive phytochemical constituents extracted from *M. oliefera* leaves is shown in Table 1. Alkaloids, saponins, tannins, polyphenols, flavonoids, carbohydrates, and glycosides were present in the aqueous extract, while terpenoid and steroid compounds were absent.

Total phenolic content and flavonoid content

The determination of total phenolic was calculated using a calibration curve of gallic acid and total flavonoid content was determined using quercetin as a standard. The total phenolic content and total flavonoid content present in the aqueous extract of *M. oleifera* leaves were 163.5 ± 4.73 mg GAE/g and 56.8 ± 7.23 mg of quercetin equivalent/g of dry powder. Results revealed that 65.25% higher phenolic content was obtained than flavonoid content from the crude leaf extract.

Synthesis and characterization of Phyto-nanocarriers: MO-AgNPs

To obtain maximum yield, the nanoparticles

Table 1. Qualitative phytochemical analysis of the aqueous leaves extract of *Moringa oleifera*. [(+) = Present and (-) = Absent]

Phytochemicals	Test	Extract
Alkaloids	Wagner's test	+
Saponins	Foam test	+
Terpenoids	Salkowski Test	-
Tannins	$FeCl_3$ test	+
Carbohydrates	Molisch test	+
Glycosides	Killani Test	+
Steroids	Salkowski Test	-

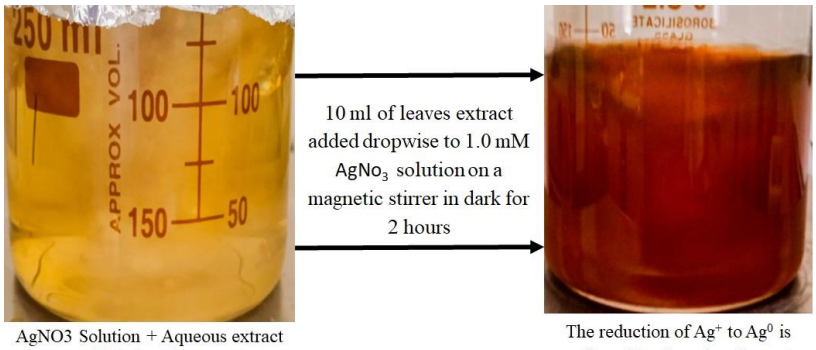

Fig. 1. Primary confirmation of the synthesis of MO-mediated Ag-Nanoparticles by the alteration of the color of the solution

synthesis route was standardized with various concentrations of *M. oleifera* leaf extract and a different volume of an aqueous solution of $AgNO_3$ (1 mM). 10 ml of leaf extract added to 100 ml 1.0 mM $AgNO_3$ produced a stable and maximum amount of silver nanoparticles. A transparent solution of $AgNO_3$ when mixed with plant leaf extract, whose color changed from pale yellow to dark yellow and finally colloidal brown, was observed within 30 min, as indicated in Fig. 1. After a few hours, there was no further change in the color of the solution indicating that the whole silver salt present in the solution had been reduced. Furthermore, the synthesis of AgNPs in solution was confirmed by UV-visible spectrophotometers for the presence of surface plasmon resonance (SPR) electrons on the nanoparticle surface. UV–vis spectra of the MO-AgNPs give a sharp peak at 428 nm after 2 hr incubation (Fig. 2A). Moreover, the stability of the nanoparticles was evaluated by analyzing Uv-Vis spectra after 24 hr, which showed an increase in the absorbance intensity to 435 nm after 24 hr of incubation (Fig. 2B). The TEM images (Fig. 3) indicate that the synthesized AgNPs were

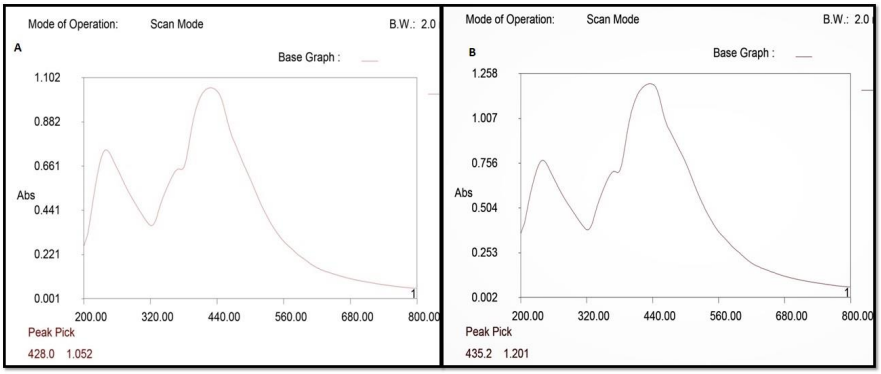

Fig. 2. A. UV-Vis Spectra of MO-AgNPs at the time of synthesis B. after 24 hr

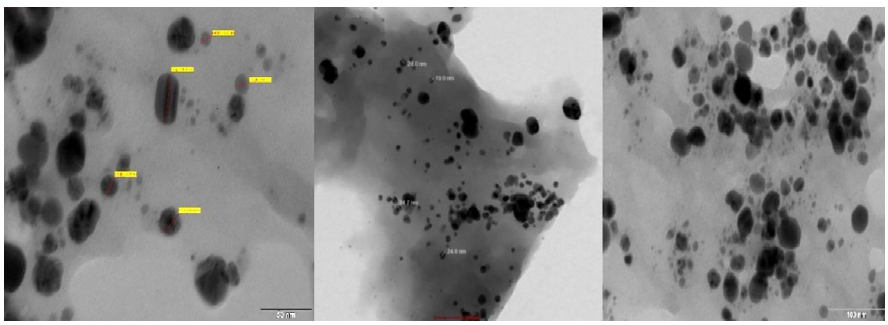

Fig. 3: Characterization of the synthesized MO-mediated AgNPs using Transmission electron microscopy (TEM)

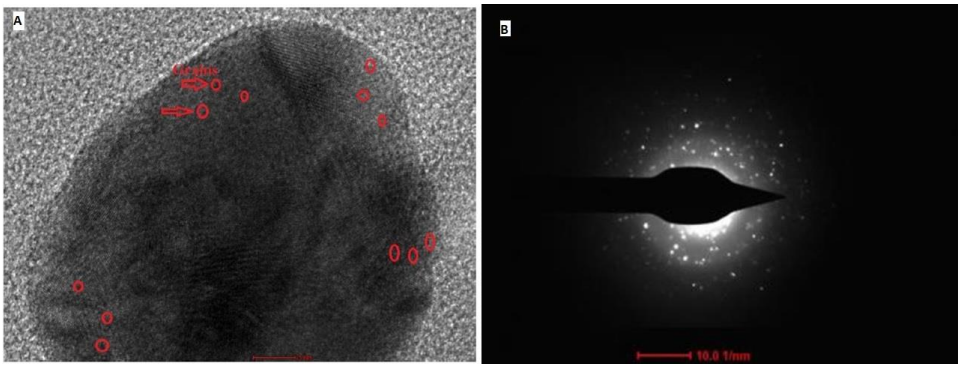

Fig. 4. A. HR-TEM images of the AgNPs showing the lattice fringes B. The HR-TEM images shows the SAED Pattern

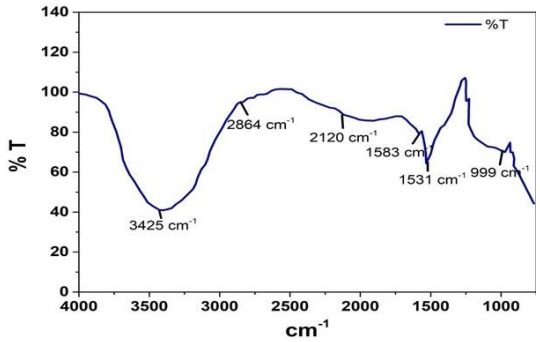

Fig. 5. FTIR graph represents the functional groups present on the surface of MO-extract mediated silver nanocarriers

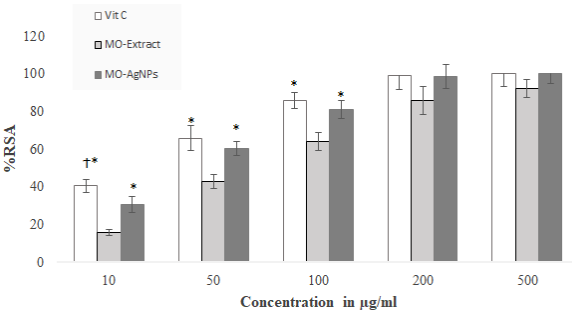

Fig. 6. DPPH radical scavenging activity of the MO-extract, MO-AgNPs and vitamin-C as a standard. The results were expressed as the mean ± SD. * *P*- value<0.05 from MO-extract and † *P*-value <0.05 from MO-AgNPs

highly mono-dispersed in spherical shapes. Apart from that, there are a few aggregates also observed at some places due to sedimentation with time. HR-TEM images (Fig. 4A) analysis revealed lattice fringes or grains on the biosynthesized silver nanoparticle particles, which indicates the crystalline nature of the MO-AgNPs. The crystallinity of the AgNPs was also confirmed by the SAED pattern shown in Fig. 4B. In addition, Fig. 5 shows the FTIR spectra of biosynthesized AgNPs derived from *M. oleifera* leaf extract. FTIR spectral data in the form of images revealed the existence of functional groups on nanocarriers which act as capping agents and are responsible for the stabilization of agents. The FTIR spectra of the synthesized silver nanoparticles displayed strong transmittance at 3425 cm^{-1}, 2864 cm^{-1}, 2120 cm^{-1}, 1583 cm^{-1}, 1531 cm^{-1}, 1031 cm^{-1}, 999 cm^{-1}. These peaks are associated with stretching vibrations of hydroxyl groups in alcohols or phenolic compounds, CH_2 and CH_3 functional groups; C=C groups of aromatic compounds or C=O groups of carboxylic acids; amide 1 ($CONH_2$) and amide 2 (CONH) groups; geminal methyl's; ether linkages and C-O or C-O-C functional groups respectively.

Anti-oxidant activity of MO-AgNPs

The anti-oxidant potential of a compound is shown by the disappearance of the violet color of free radical DPPH followed by a decline in optical density measured at 517 nm. Fig. 6 exhibits anti-oxidant capacity in terms of percent radical scavenging activity of crude *M. oleifera* leaf extract, MO-AgNPs, and standard ascorbic acid with five different concentrations (10, 50, 100, 200, and 500 µg/ml). The data indicates a significant ($P<0.05$) dose-dependent increase in the percent radical scavenging activity. The free radical scavenging activity was noted to be significantly ($P<0.05$) higher in *M. oeifera* mediated nanoparticles (30.5%, 60.5%, 80.9%, 98.7%) when compared to the aqueous extract of *M. oleifera* leaves (15.7%, 42.8%, 63.8%, 85.8%) up to 200 µg/ml. Plant leaf extract with a higher concentration (500 g/ml) has the highest anti-oxidant potential, similar to MO-AgNPs. The most remarkable observation noted that the *M. oeifera* mediated silver nanoparticles expressed anti-oxidant activity equivalent to the standard anti-oxidant vitamin C at all studied concentrations except 10 µg/ml.

Anti-arthritic assay

Three different concentrations (100, 200, 500 µg/ml) of the diclofenac sodium as a positive

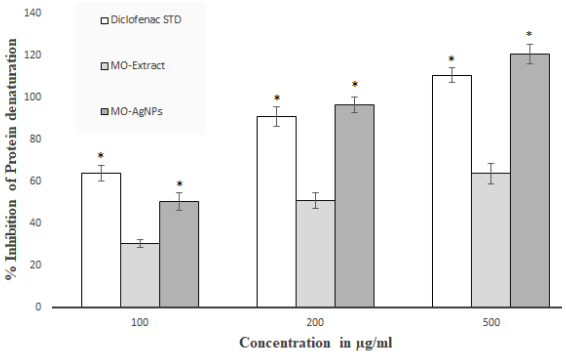

Fig. 7. Inhibition of protein denaturation as a means of *in vitro* anti-arthritic activity of MO-extract, MO-AgNPs and Diclofenac sodium as a standard. The results were expressed as the mean ± SD. * *P*-value <0.05 from MO-extract

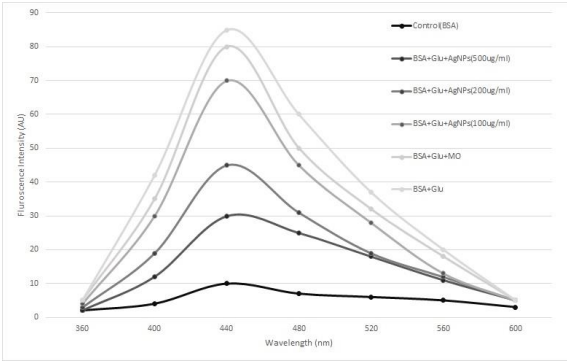

Fig. 8. Protein fluorescent absorbance of BSA as a Control, BSA+ glucose, BSA+ glucose+ MO-extract as well as BSA+ glucose + different combinations of the MO-AgNPs

control, crude leaves extract, and MO-AgNPs were used for the inhibition of protein denaturation assay to determine antiarthritic activity. The result of the anti-arthritic assay is presented in Fig. 7. *M. oleifera* mediated nanoparticles significantly ($P<0.05$) display an 89.16% higher protective effect on protein denaturation compared to aqueous leaves extract. Diclofenac sodium demonstrated 110.4% protection against heat-induced structural alterations in bovine serum albumin protein. The result of antiarthritic activity revealed that MO-AgNPs demonstrate more significantly ($P<0.05$, 9.15%) stave off protein denaturation than standard drug diclofenac sodium.

In vitro anti-glycation assays using the bovine serum albumin (BSA-glucose) model

The development of fluorescent AGEs in glucose-induced protein glycation was determined by monitoring their fluorescent intensity at the end of 4 weeks of incubation of protein BSA with glucose. As displayed in Fig. 8, a significant increase in fluorescent intensity in BSA incubated with glucose was noted during 4 weeks of incubation indicating the level of glycated BSA increased over a period 4 weeks. However, in the presence of MO-AgNPs with 500 µg/ml, fluorescent intensity was most significantly declined by 65% compared to control. Table 2 shows the antiglycation effect of biologically synthesized MO-AgNPs on glycated albumin measured in the form of fructosamine. Fructosamine level was formed in the glucose-BSA system measured as an index of glycation formed. At the end of week 4, the presence of MO-AgNPs (100-500 µg/ml) effectively diminish the level of fructosamine (15.89%-84.56%) compared to

Table 2. The percentage of glycation inhibition exhibited by *Moringa oleifera* leaves extract, MO-mediated AgNPs and aminoguanidine as control at different concentrations (Mean ± SD, n=5)

Treatment	Conc. (µg/ml)	Inhibition of AGEs formation (%)
Control (Aminoguanidine)	100	16.05 ± 0.82
	200	65.61 ± 2.74
	500	90.81 ± 3.22
Moringa leaves Extract	100	10.08 ± 1.64
	200	25.65 ± 3.78
	500	60.25 ± 5.30
MO-AgNPs	100	13.89 ± 1.20*
	200	50.80 ± 4.92**
	500	84.56 ± 4.41**

leaves extract (10.8%-60.5%). The potential of MO-AgNPs in inhibiting the formation of fructosamine was found to be equally effective as standard antiglycation agent aminoguanidine.

DISCUSSION

In the present study, silver nanocarriers were synthesized from aqueous leaf extract of *M. oleifera* and also investigated their anti-oxidant, anti-arthritic, and inhibitory effects on protein glycation. The result revealed that MO-AgNPs efficiently inhibited the formation of fluorescent and non-fluorescent AGEs. A significant decline in the protein denaturation in presence of MO-AgNPs indicates great potential as an antiarthritic agent. Water as the solvent selected for the extraction of bioactive phytochemicals from the leaves of *M. oleifera* due to its higher polarity index compared to another solvent for the extraction of various active plant secondary metabolites like phenolics and flavonoids. The phenolic compounds in plants contain the hydroxyl group, which acts as a good scavenger and is known as a prevailing chain-breaking anti-oxidant (26). Higher content TPC and TFC values of Moringa leaves found in this study were similar to those of moringa samples reported by Vyas (27). It is well established that the amount of phenolic and flavonoids directly affects the anti-oxidant potential of plant extract. Generally, AgNPs are formed by the reduction of silver salt to silver ions (Ag+ to Ag0) (28). The various phytochemicals in plant extracts, such as alkaloids, terpenoids, and flavonoids, act as reducing agents responsible for the reduction of silver ions to AgNPs (29). The synthesis of MO-AgNPs was optimized by variable reaction parameters such as time and the concentration of both $AgNO_3$ and the plant extract solutions. In an aqueous solution, silver (nanoparticles exhibit strong surface plasmon resonance (SPR) (30). The FTIR analysis is carried out for the evaluation or the identification of various phytochemical groups present in the extract, which are bound specifically to the silver surface and are responsible for the synthesis and stabilization of silver nanoparticles by acting as a reducing and capping agent. From the FTIR analysis, it's also known that carbonyl groups of amino acid residues have a very strong binding affinity with silver nanoparticles, and they act as reducing and stabilizing agents to avoid agglomeration and give stability to the silver nanoparticles. The amide 1 and amide 2 groups observed at 1641 cm^{-1} are responsible for the reduction of metal ions and stabilization processes. This peak indicates the presence of some bio-organic compounds such as terpenoid groups (31). FTIR spectra indicate the presence of characteristic functional groups such as alcohols, aldehydes, flavonoids, phenols, and nitro compounds as phytoconstituents present in the leaves of *M. oleifera leaves,* which participate in the bio-reduction process for the synthesis of silver nanoparticles. The free radical DPPH is extensively applied during the assessment of anti-oxidant properties in-vitro. The radical scavenging activity (RSA) of MO-AgNPs was compared with ascorbic acid used as a reference. The lower IC_{50} value of MO-AgNPs indicates greater anti-oxidant potential compared with the aqueous extract of moringa leaves. The higher content of phenolic and flavonoid compounds present in *M. oleifera* leaves act as primary anti-oxidants (32). The present result shows enhanced anti-oxidant potential in MO-AgNPs when compared with crude extract of moringa leaves, possibly due to synergistic mechanisms that increased anti-oxidant cascade mechanisms. Previously, it was reported that the synthesis of Ag-NPs from plant extract increased total anti-oxidative and antimicrobial efficiency more than the plant extract alone or $AgNO_3$ (33). The absorbance of *M. oleifera* leaf extract, MO-AgNPs, and reference drug (diclofenac sodium) concerning control indicates stabilization of the protein. The *M. oleifera* leaf extract and MO-AgNPs exhibit higher anti-arthritic activity at various concentrations (100, 200, and 500µg/ml) by protein denaturation inhibition. Investigators reported the anti-inflammatory effect of *MO* leaves extract may be due to the reduction of the inflammatory process by inhibiting the action of neutral factor kappa-beta (NF-kβ) and subsequent NF-kB-dependent downstream events and inflammation (34). So, in the above study, it is assumed that the synthesized silver nanoparticles from *M. oleifera* leaf extract can regulate the production of auto-antigens and inhibit the denaturation of protein and membrane lysis in RA participants. It is well observed that the non-enzymatic glycation of protein plays a critical role in the development of arthritis pathogenesis and is also involved in accelerating the progression of arthritis (35). Concurrent modification of albumin by reactive oxygen species and dicarbonyls generates glycated albumin, which may persist in the circulation for a longer duration and contribute to various forms

of arthritis. Several studies highlight elevated levels of AGEs in serum and urine, reflecting the increased disease activity in RA patients (36). In the present investigation, the total amount of glycated protein formed during the experiment was assessed by the fluorescence measurement and the formation of fructosamine for the incubation period of 3 weeks. It is well documented that AgNPs significantly inhibit AGEs formation in a concentration-dependent manner (37). Previous studies have shown inhibitory effect of phytoconstituents extracted from *M. oliefera* leaf on the development of different types of advanced glycation end-products (AGEs) in vitro (38). However, in the presence of MO-AgNPs, while the concentration increased, the fluorescence intensity and fructosamine levels were found to decline, and the inhibitory effect was observed to follow the order of 100 < 200 < 500 µg/ml. This observation suggests that the addition of phytoconstituents on the surface of silver nanoparticles can efficiently reduce the glycation of protein and also prevent its accumulation in the tissue. The inhibitory effect of plant extract and MO-AgNPs can be attributed to the presence of various anti-oxidant compounds. The results were similar to previous studies reported on glucose-treated BSA. MO-AgNPs' synergistic effects suggest that the presence of different phytochemicals, specifically phenolic and flavonoid compounds, may be responsible for their potent inhibitory effects at an earlier stage of protein glycation. The presence of flavonoids, saponins, and alkaloids in aqueous extracts of the leaves may explain the possible mechanism for the anti-arthritic properties of this plant (39). *Invitro* bioassay results of the antiarthritic effect of AgNPs assessed against denaturation of albumin revealed that all tested concentrations significantly (P≤0.001) inhibited thermally egg albumin denaturation with a maximum percentage of Diclofenac, used as a standard drug, induced 90.8 % inhibition at 200µg/ml concentration.

CONCLUSION

The present study demonstrates that the green synthesis of silver nanoparticles using the aqueous leaf extract of *Moringa oleifera* is simple and eco-friendly. The leaf extract contains phenolic and flavonoid compounds which might be responsible for the bio-reduction of silver ions and the capping/stabilization of the silver nanoparticles, as confirmed by FTIR studies. Overall, the result highlights the effectiveness and potential application of AgNPs in biomedical fields such as in drug formulation for treating anti-arthritic and anti-inflammatory-related disorders. Plant-based bioactive compounds have shown significant promise in the prevention and treatment of a variety of chronic diseases, including type 2 diabetes, cardiovascular disease, arthritis, hepatic disease, and cancer. However, their clinical uses have been limited due to their poor water solubility, low stability in-vivo, low absorption, and low bioavailability. Hence, nanocarriers are employed to overcome such barriers to improve solubility and stability of phytoconstituents, enhance their absorption and reduce their toxicity, which is achieved by reducing their particle sizes and by modifying their surface properties. Nanocarriers have thus provided crucial steps in bringing medicinal phytoconstituents closer to their clinical demand. However, additional studies are required to assess and improve the long-term safety of nano-sized phytoconstituents to further enhance their use in biomedicine. This finding shows that *Moringa Oleifera* leaves mediated silver nanoparticles are capable of controlling protein denaturation in the arthritic cells. Thus, *Moringa Oleifera* leaves could be promising for the development of novel plant-based anti-arthritis drugs.

ACKNOWLEDGEMENTS

We thank to Sophisticated Instrumentation Centre for Applied Research and Testing - SICART for their assistance in the analysis and characterization of nanoparticles.

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