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Research Article

Antiarthritic activity of synthesized silver nanoparticles from aqueous extract of *Moringa concanensis* Nimmo leaves against FCA induced rheumatic arthritis in rats

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ABSTRACT

Background and Objective: The rheumatoid arthritis is autoimmune disease and it is a major health issues among the peoples in worldwide. People affected by rheumatism have double risk of heart disease, lungs and chest infections, which causes 10-20% of deaths in individuals. The present research work was aimed to evaluate the antiarthritic potential of synthesized silver nanoparticles from the aqueous extract of *M.concanensis* leaves.

Material and Methods: The arthritis was induced by complete Freund's adjuvant method and the rats was treated with synthesized silver nanoparticles from aqueous extract of *M.concanensis* leaves. The body weight, paw volume, hematological parameters, liver function marker enzymes and the renal function markers of the arthritic rats and nanoparticles treated rats was analyzed by the standard methods.

Results: The results of the present study were made the new findings in antiarthritic activity of silver nanoparticles from *M.concanensis* leaves. The nanoparticles showed a noteworthy antiarthritic activity against FCA induced rheumatoid arthritis in experimental rats.

Conclusion: Based on the above results, it was concluded as the present work are states the better understand on the antiarthritic activity of silver nanoparticles of *Moringa concanensis* Nimmo leaves.

Keywords: *M.concanensis* leaves, silver nanoparticles, rheumatoid arthritis, FCA, diclofenac and Wistar rats

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1. INTRODUCTION

Rheumatoid arthritis is an autoimmune disease with joint inflammation, synovial proliferation and destruction of articular cartilage and the majority is seen in female. Prolonged treatment with allopathic drugs causes adverse side effect and hence alternative natural measure is in search¹. In the year of 2007, arthritis foundation has projected that two third of the population was suffering from arthritis and census reports that by 2030, this was increases beyond to 40%². People affected by rheumatism have double risk of heart disease, lungs and chest infections, which causes 10-20% of deaths in individuals. Inflammation was the important pathological change of rheumatoid arthritis³. The inefficiency of renal function is directly associated with the development of joint pain. The management of rheumatoid arthritis is a multidisciplinary approach in order to reduce the pain, decreases of inflammation and restoration of joints function. However,

progresses in understanding the pathogenesis of the disease have encouraged the development of new approachable therapeutics, with enhanced outcomes⁴. In practical terms suppression of inflammation is the target intensive therapy. Herbal medicines have become popular for the treatment of rheumatoid arthritis in worldwide recently⁵⁻⁷.

The applications of silver nanoparticles on different areas like medicines, chemistry, catalysis and electronics are well known⁸. The production of silver nanoparticles can be done by physical methods, chemical methods and biological methods. Among these methods, the biological methods of silver nanoparticles synthesis are eco-friendly; no toxic or harmful chemicals were utilized for the production of silver nanoparticles^{9,10}. In this method, the plant extracts, enzymes, proteins, antioxidants, triglycerides, saponins, glycoproteins, polysaccharides, terpenes, flavonoids, and tannins are used for the reduction and stabilization of nanoparticles¹¹. The present study deals with the evaluation the antiarthritic

activity of synthesized silver nanoparticles from *M.concanensis* leaves in experimental rats.

2. MATERIAL AND METHODS

2.1 Collection and identification of plant

The healthy, matured and insect bites free leaves of *Moringa concanensis* Nimmo plant (Family - *Moringaceae*) was collected from Esanai village, Perambalur district, Tamilnadu, India (Latitude - 11.2982° N, Longitude - 78.8298° E). The plant sample was identified and authenticated by Dr. C. Murugan, Scientist, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The identification number BSI/SRC/5/23/2016/Tech-152.

2.2 Synthesis of silver nanoparticles

2.2.1 Preparation of plant extract

The 10 gms of the dried *M.concanensis* Nimmo leaves powder were kept in a beaker containing 100 ml double distilled water and boiled at 80°C for 10 minutes to obtain bioactive compounds from *Moringa concanensis* Nimmo leaves. The extract was cooled and filtered through normal filter paper followed by Whatmann filter paper No.1. The final extract was used to synthesize silver nanoparticles.

2.2.2 Synthesis of silver nanoparticles

The aqueous solution of silver nitrate (AgNO_3) at concentration of 0.001 M was prepared to synthesize silver nanoparticles. The 5 ml *Moringa concanensis* Nimmo leaves aqueous extract was slowly added to 95 ml of aqueous solution of AgNO_3 while stirring. The formation of reddish brown color was observed after 3 hrs incubation at room temperature. Then the AgNPs solution was purified by repeated centrifugation at 10,000 rpm for 20 minutes. After centrifugation the obtained particles were washed with distilled water for 10 to 20 minutes and kept in hot air oven for drying at 100°C for 1 hour.

2.2.3 Characterization of silver nanoparticles

2.2.3.1 UV-Visible spectroscopy analysis

The formation and stability of silver nanoparticles in aqueous solution was confirmed by UV-Visible spectrophotometer analysis. The reduction of pure Ag^+ ions was monitored by measuring the UV-Visible spectrum of the reaction medium at 540 nm for 12 hours. UV-Visible spectral analysis was done by UV-Visible spectrophotometer (UV-2450, Shimadzu).

2.2.3.2 Fourier transform - Infra red analysis (FT-IR)

The FT-IR spectroscopy measurements are carried out to identify the biomolecules that bound specifically on the silver surface and local molecular environment of capping agent on the nanoparticles. To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 10,000 rpm for 10 mins and the resulting suspension was redispersed in 10 ml sterile distilled water. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FT-IR spectrophotometer.

2.3 Selection of animals

Healthy adult male wistar albino rats weighing about 150 to 200g were obtained from Viprogen Bioscience Private Limited, Mysore. They were housed in polypropylene cages under the standard laboratory condition ($25 \pm 2^\circ\text{C}$, humidity 60-70%, 12 hours light / dark cycles). The rats were fed with

commercial rat pellet diet and water was provided *ad libitum*. The rats were acclimatized to laboratory conditions for one week prior to the commencement of the experiments. The animal care and handling were done according to the regulations of council directive of committee for the purpose of control and supervision of experiments on animals (CPCSEA) on animal experimentations. The clearance No: VIP/IAEC/8/2016). All animal experiments were performed in the laboratory according to the ethical guidelines suggested by the International Animal Ethics Committee (IAEC).

2.4 Preliminary animal studies in toxicity studies of dose fixing for silver nanoparticles

2.4.1 Experimental design

Group I : Normal rats (Standard diet)Gr

Group II : Rats fed orally with silver nanoparticles of *Moringa concanensis* Nimmo 50 $\mu\text{g}/\text{kg}$ body weight for 14 days.

Group III : Rats fed orally with silver nanoparticles of *Moringa concanensis* Nimmo 100 $\mu\text{g}/\text{kg}$ body weight for 14 days.

Group IV : Rats fed orally with silver nanoparticles of *Moringa concanensis* Nimmo 150 $\mu\text{g}/\text{kg}$ body weight for 14 days.

Group V : Rats fed orally with silver nanoparticles of *Moringa concanensis* Nimmo 200 $\mu\text{g}/\text{kg}$ body weight for 14 days.

They were continuously observed for 4 hrs to detect any changes in the behavior in relation to the posture, mood and motor activity.

2.4.2 Induction of arthritis

Arthritis was induced by 0.1 ml injection of Freund's complete adjuvant emulsion (FCA) into the sub-planter surface of right hind paw¹².

2.4.3 Experimental design for evaluation of antiarthritic activity

After one week of acclimatization period, the rats were divided into four groups with six rats in each.

Group I : Control rats fed with standard diet and water *ad libitum*.

Group II: Arthritis induced rats received 0.01 ml of FCA intradermally.

Group III : Arthritis induced rats received silver nanoparticles of *M.concanensis* Nimmo leaves (150 $\mu\text{g} / \text{kg}$ body weight) by i.p administration for 28 days.**Group I**

V : Arthritis induced rats received Diclofenac (standard drug) 10 mg / kg body weight by oral administration for 28 days.

2.5 Collection of samplesAfter the experimental regimen (4 weeks), the rats were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected in EDTA coated centrifuge tubes by an incision made in the jugular veins and serum was separated by centrifugation at 2000 rpm for 20 minutes and utilized for various biochemical assays. The liver and hind limb were excised immediately and thoroughly washed with ice cold physiological saline and blotted dry. A part of the tissues such as liver and hind limb were removed and fixed in 10% formalin for histopathological studies.

2.5.1 Body weight

The initial and final body weight of each rat was assessed using a sensitive balance and recorded.

2.5.2 Measurement of paw volume

The mean increase in the paw volume of each group was measured the initial and final days using digital plathysmograph (Ugobasile, Italy).

2.5.3 Hematological parameters

The haematological parameters such as haemoglobin, PCV, WBC, RBC and Platelets were assayed. The whole blood sample was analysed for the changes in the blood cells using SYSMEX Xs – 800 i automatic haematology analyzer.

2.5.4 Estimation of erythrocyte sedimentation rate (ESR)

To determine the rate at which the red blood cells sediment by the Westergren's Method. Collect 1.6 ml blood in a plain acid washed glass tube containing 0.4 ml of 3.8% sodium citrate till the minimum fill level indicated on the label. If K₂EDTA blood is used, add 0.5 ml of 3.8% sodium citrate to 2 ml of K₂EDTA blood. Mix the blood with sodium citrate solution immediately by gently inverting the tube at least 6 times. Place the Westergren's tube up right and exactly vertical in the stand, note the time, allow standing exactly for 1 hour, and note the level to which the red blood cell has settle down at the end of 1 hour.

2.5.5 Assay of hematological parameters

After induction of rheumatoid arthritis blood sample were collected from infected wistar albino rats. The hematological parameters like Hb, PCV, WBC, RBC, Platelets and ESR were assayed. The blood samples were analysed for the changes in the blood cells using hematology analyser.

2.6 Estimation of liver marker enzymes

2.6.1 Estimation of aspartate transaminase

In different test tubes, 1 ml of buffered substrate was added to 0.1 ml of serum or 0.1 ml of liver homogenate and incubated at 37°C for 1 hr. Then 1 ml of DNPH reagent was added to arrest the reaction. Then the blank tubes 0.1 ml of distilled water was added instead of serum. The tubes were kept inside for 15 minutes, and then 10 ml of 0.4 N sodium hydroxide was added and read at 520 nm in UV spectrophotometer. The enzyme activity was expressed as IU/litre for serum and micromole of pyruvate liberated/hour/mg protein for liver homogenate.

2.6.2 Estimation of alanine transaminase

0.2 ml of sample, added 1 ml of the buffered substrate and incubated for 30 minutes at 37°C. The control tubes, enzyme was added after arresting the reaction with 1 ml of DNPH and the tubes was kept at room temperature for 20 minutes. Then 10 ml of 0.4N NaOH was added. A set of standard pyruvate in the concentration 0.4 to 2 μ m was also treated in a similar manner. The colour developed was read at 520 nm. The enzyme activity was expressed as μ moles of pyruvate liberated per litre in serum.

2.6.3 Estimation of alkaline phosphatase

Pipetted out 4.0 ml of the buffered substrate into a test tube and incubated at 37°C for 5 minutes. 0.2 ml of serum was added and incubated for 15 minutes. Removed and immediately added 1.8 ml of diluted phenol reagent. A control was run simultaneously with 4 ml buffered substrate and 0.2 ml sample to which 1.8 ml phenol reagent was added

immediately. Mixed well and centrifuged. 4 ml of the supernatant added 2 ml of sodium carbonate. Standards were also run. Incubated all the tubes at 37°C for 15 minutes, the colour developed were measured at 700 nm. The enzyme activity was expressed as μ moles of phenol per litre in serum.

2.6.4 Estimation of acid phosphatase

Pipetted out 4 ml of the buffered substrate into a test tube and incubated at 37°C for 5 minutes. Then add 0.2 ml of sample and incubated for 60 minutes and removed then immediately added 1.8 ml of diluted phenol reagent. At the same time a control was set up containing 4 ml buffered substrate and 0.2 ml of sample to which 1.8 ml of phenol reagent was added immediately. Mixed well and centrifuged. 4 ml of the supernatant added 2 ml of sodium carbonate. Take 4 ml of working standard solution and for blank taken 3.2 ml water and 0.8 ml of phenol reagent. Then add 2 ml of sodium carbonate. All the tubes were incubated at 37°C for 15 minutes. Read the colour development at 700 nm. The activity of serum acid phosphatase was expressed in μ moles of phenol liberated per litre. The activity in tissue homogenate was expressed as μ moles of phenol liberated/min/mg protein.

2.6.5 Estimation of lactate dehydrogenase

Place 1 ml buffered substrate and 0.1 ml sample into each of two tubes. Add 0.2 ml of water to the blank. Then to the test add 0.2 ml of NAD. Mixed and incubated at 37°C for 15 minutes. Exactly after 15 minutes, 1 ml of dinitrophenyl hydrazine was added to each (test and control). Tube acclimatized for 15 minutes, and then added 10 ml of 0.4N sodium hydroxide and the colour developed was read immediately at 440 nm. A standard with sodium pyruvate solution with the concentration range 0.1 - 1 μ mole was taken. LDH activity in serum was expressed as micromoles of pyruvate liberated per litre.

2.6.6 Estimation of γ -glutamyl transferase (γ -GT)

0.5 ml of serum was added to the incubation mixture containing 0.5 ml gamma-glutamyl p-nitroanilidine, 2 ml glycyl glycine and 1 ml buffer. After incubation for 30 minutes at 37°C the reaction was arrested by the addition of 1 ml of 10% acetic acid.

The amount of p-nitroaniline liberated in the supernatant was measured as the difference in optical density at 410 nm, between samples with and without substrate. The substrate incubated in the absence of serum under the same condition was used as a reference blank. Enzyme activity was expressed as IU/l for serum.

2.6.7 Estimation of glucose

The 0.1 ml of blood was mixed with 1.9 ml of Tricorboxylic acid solution to precipitate protein and then centrifuged. 1 ml of the supernatant was mixed with 4 ml of O-toluidine reagent and was kept in a boiling water bath for 15 minutes and the green colour developed was read at 600 nm, in UV spectrophotometer. A set of standard glucose solution were also treated similarly. The value was expressed as mg/dl blood.

2.6.8 Estimation of total cholesterol

10 ml of ferric chloride – uranyl acetate reagent was added to 0.1 ml of sample (serum), mixed well and allowed to stand for 5 minutes and centrifuged. 3 ml of the supernatant was taken for the analysis. Similarly 0.1 ml of standard cholesterol was mixed and 3 ml of aliquot was taken. Blank tubes contained 3 ml of ferric chloride – uranyl acetate

reagent 2 ml of ferrous sulphate – sulfuric acid reagent was added to all the tubes and mixed well. The colour intensity was measured at 540 nm after 20 minutes in a UV visible spectrophotometer. The serum cholesterol was expressed as mg/dl.

2.6.9 Estimation of triglycerides

The 4 ml of isopropanol was added to 0.1 ml of sample (serum) and mixed well, followed by 0.4 g of alumina and shaken well for 15 minutes. Centrifuged at 2000 rpm for 10 minutes and then 2 ml of the supernatant fluid was transferred to appropriately labeled tubes. The tubes were placed in a water bath at 65°C for 15 minutes for saponification after adding 0.6 ml of the saponification reagent. After adding 1ml of sodium meta periodate was added followed by 0.5ml of acetyl acetone reagent. After mixing the tubes were kept in a water bath 65°C for half an hour. The contents were cooled and the absorbance was measured at 430 nm against a blank in a UV-visible spectrophotometer. Triglyceride content of the serum was expressed as mg/dl.

2.7 Estimation of renal function markers

2.7.1 Estimation of urea

The 0.1ml of blood was added to 3.3ml of water and mixed with 0.3 ml of 10% sodium tungstate and 0.3 ml of 0.67 N sulfuric acid reagents. The suspension was centrifuged and to 1ml of the supernatant 1ml of water, 0.4ml of diacetyl monoxime and 2.6 ml of sulfuric acid – phosphoric acid reagents were added in that order and kept in a boiling water bath for 30 minutes. This was cooled and the colour developed was measured at 480 nm in a UV-visible spectrophotometer. Aliquots of urea were also treated in a similar manner. The value was expressed as mg/dl blood.

2.7.2 Estimation of creatinine

A protein free filtrate was prepared by precipitating a serum with 8 ml of water, 0.5 ml of 3N sulfuric acid and 0.5 ml of 10% sodium tungstate. After centrifugation, 5 ml of the clear filtrate was taken. Then this was added 1.5 ml saturated picric acid solution and 1.5 ml of 0.75 N sodium hydroxide. The colour intensity was measured at 470 nm after 15 minutes. Standard and blank were also processed similarly. Serum creatinine levels were expressed as mg/dl.

2.7.3 Estimation of uric acid

0.1 ml of the sample, 2.9 ml of water was added, followed by 0.6 ml each of phosphotungstic acid and sodium carbonate. A blank was set up with 3 ml water. Standards were also treated in the same manner. The colour was read at 640 nm after 10 minutes. The result was expressed as mg / dl in serum.

2.7.4 Estimation of protein

Pipetted out 1.0 ml of working standard solution, 0.1 ml of the sample was taken. The volumes in all the tubes were made upto 1 ml with distilled water. Add 5 ml of alkaline copper reagent to each tube. Mixed well and allowed to stand for 10 minutes. Then add 0.5 ml of Folin-Ciocalteu reagent. Mixed well and incubated at room temperature for 30 minutes. A reagent blank was also prepared. After 30 minutes, the blue colour developed was measured at 660 nm.

2.7.5 Estimation of albumin

Take three test tubes labeled B (blank), S (standard) and T (test). The blank solution contains 1000 µl of reagent and 10 µl of distilled water. The standard solution contains 1000 µl

of reagent with 10 µl of standard solution. The test solution contains 1000 µl of reagent with 10 µl of sample solution. Set the instrument to zero with reagent blank. Mixed thoroughly incubated test tube at room temperature for 3 minutes. Read absorbance at 630 nm or red filter (620–650 nm). The values were expressed in g / dl in serum.

2.7.6 Estimation of C – Reactive protein (CRP)

Pipetted out the 990 µl of wash buffer into a test tube. Add 10 µl of the provided standard (1:100 dilution) to create the top standard, 10 ng / ml. Dilute samples as necessary in wash buffer. Serum sample should be diluted at least 1:4,000 prior to assay. Add 100 µl of standard or sample per well of the microwell plate. Record locations of each addition for later reference. Seal plate with an adhesive strip and incubate at room temperature for 2 hrs. Dilute the HRP-conjugated antibody 1:100 in wash buffer. 9.9 ml of wash buffer, add 100 µl of stock conjugate. Add 100 µl of the diluted conjugate to each well of the plate. Seal and incubate for 1 hour at room temperature. Each well, add 100 µl of the TMB substrate solution. A blue colour indicates a positive reaction. Allow reaction to proceed at room temperature for 5-10 minutes. The reaction was stopped by adding 100 µl of stop solution per well. The reaction mixture should turn to yellow. Read the absorbance (OD) on an ELISA plate reader equipped with a 450 nm filter. If wavelength correction is available, set to 570 nm. Readings made without wavelength correction may be higher and less accurate. Use the standard curve to determine the amount of CRP present in the samples. The concentration read from the curve should be multiplied by the dilution factor for any diluted samples.

2.8 Histopathological investigation of hind limb and liver

The hind limb and liver was removed, washed with ice cold saline and a small portion of this was quickly fixed in 10% formalin.

2.8.1 Tissue processing

The tissues were placed in 10% formal saline (10% formalin in 90% sodium chloride) for one hour to rectify shrinkage due to higher concentration of formalin. The tissue was dehydrated by ascending grades of isopropyl alcohol by immersing in 80% isopropanol overnight, 100% isopropyl alcohol for 1 hour. The dehydrated tissues were cleared in two changes of xylene, 1 hour each. Then the tissues were impregnated with histology grade paraffin wax (melting point 58 - 600°C) at 60°C for 2 changes of 1 hour each. The wax impregnated tissues were embedded in paraffin blocks, mounted and cut with rotary microtome at 3 micron thickness. The sections were floated on tissue floatation bath at 40°C and taken on glass slides and smeared with equal parts of egg albumin and glycerol. The sections were then melted in an incubator at 60°C and after 5 minutes and the section were allowed to cool.

2.8.2 Tissue staining

The sections were deparaffinised by immersing in xylene for 10 minutes in horizontal staining jar. The deparaffinised section were washed with 100% isopropyl alcohol and stained in Ehrlich's hematoxylin for 8 minutes in horizontal staining jar. After stained in hematoxylin, the sections were washed with tap water and dipped in acid alcohol to remove excess stain (8.3% HCl in 70% alcohol). The sections were then placed in running tap water for 10 minutes for blueing (show alkalization). The section were counter stained in 1% aqueous eosin (1 g in 100 ml tap water) for one minute and the excess stain was washed with tap water and the sections were allowed to dry. Complete dehydration of stained

sections was ensured by placing the section in the incubator at 600°C. When the sections were cooled, they were mounted in DPX mount having the optical index of glass (the section were wetted in xylene and inverted on to the mountant placed on cover slip). The architecture was observed at low power objective. The liver cell injury and other aspects were observed under high power dry objective.

2.9 Statistical analysis

The values were expressed as Mean \pm SD. Significant difference have been observed using One Way Analysis of Variance (ANOVA) by SPSS software version 19.0. The $p < 0.05$, was considered as significant difference.

3. RESULT

3.1 Biosynthesis of silver nanoparticles from *Moringa concanensis* Nimmo leaves

3.1.1 Visual observation

The silver nanoparticles were synthesized from aqueous extract of *Moringa concanensis* Nimmo leaves. The formation of silver nanoparticles from reaction medium was confirmed by colour change. The reaction mixture contains the aqueous extract of *M.concanensis* Nimmo leaves and aqueous silver nitrate solution. After the 24 hrs of dark incubation the colour of the reaction medium was changed from light brown to black. This result indicates the formation of silver nanoparticles by the reduction of Ag ions.

3.1.2 UV-Visible Spectroscopy analysis of synthesized silver nanoparticles from aqueous extract of *M.concanensis* Nimmo leaves

The UV spectrum showed the surface plasma AgNPs at increasing concentration was taken and the colour changes were observed for nanoparticles. For silver colour changes from colourless to dark brown colour. Metal nanoparticles can be synthesized by reducing metal ions using some chemical molecules. In green synthesis, this is observed that natural material extract act as reducing agent for generation of metal nanoparticles.

3.1.3 Fourier transform – infrared red (FT-IR) analysis of synthesized silver nanoparticles from aqueous extract of *M.concanensis* Nimmo leaves

The FT-IR spectroscopy measurements were carried out to identify the biomolecules that bound specifically on the silver surface and local molecular environment of capping agent on the nanoparticles. The synthesized silver nanoparticles were analyzed by FT-IR spectrophotometer. The FT-IR spectra of biosynthesized AgNPs from *M.concanensis* Nimmo leaves extract, showed in figure 15, indicates the presence of carboxylic, hydroxyl, alkanes, alkenes and carbonyl groups. Display of strong broad O–H stretch carboxylic bands in the region 3273.34 cm^{-1} and carbonyl stretching bands in the region 1070.54 cm^{-1} are observed. The peaks appearing in the region 1630.88 cm^{-1} are attributed by the alkene group. The alkanes stretching bands was observed in the region of 2847.05 cm^{-1} and 2915.53 cm^{-1} in FT-IR spectrum.

3.2 Effect of Silver nanoparticles of *Moringa concanensis* leaves on hematological parameters in blood of experimental rats

For the dose fixation studies on the anti-rheumatoid arthritis efficiency of synthesized silver nanoparticles from aqueous leaves extract of *Moringa concanensis* Nimmo against freund's complete adjuvant (FCA) intoxicant, we focused on the effect of silver nanoparticles from aqueous leaves extract of *Moringa concanensis* Nimmo on hematological parameters in the blood of experimental rats. The dose fixation study was employed in various concentration of silver nanoparticles i.e., 50 $\mu\text{g}/\text{kg}$ b. w to 200 $\mu\text{g}/\text{kg}$ of body weight. The table 1 showed that there was a no significant ($p < 0.05$) changes in hemoglobin, packed cell volume (PCV), white blood cells (WBCs), red blood cells (RBCs) and platelets with EEMC up to 150 $\mu\text{g}/\text{kg}$ of body weight in relation with control group. However 200 $\mu\text{g}/\text{kg}$ b.w of silver nanoparticles treated rats showed a significant ($p < 0.05$) decrease in relative blood components. The significant ($p < 0.05$) decreases in these blood components may due to the higher doses of silver nanoparticles. Hence the 150 $\mu\text{g}/\text{kg}$ concentration was selected as optimum dose (table 1).

Table 1: Effect of silver nanoparticles on hematological parameters

Experimental groups	Hb (g %)	PCV (%)	WBC ($10^3/\mu\text{l}$)	RBC ($10^{12}/\mu\text{l}$)	Platelets ($10^9/\mu\text{l}$)
Group I (Control)	12.6 \pm 0.30	37.50 \pm 0.81	6.50 \pm 0.40	5.80 \pm 0.30	5.24 \pm 0.30
Group II (50 $\mu\text{g}/\text{kg}$ b.w)	12.6 \pm 0.20	37.50 \pm 0.60	6.40 \pm 0.50	5.75 \pm 0.40	5.23 \pm 0.50
Group III (100 $\mu\text{g}/\text{kg}$ b.w)	12.5 \pm 0.30	37.50 \pm 0.50	6.50 \pm 0.40	5.80 \pm 0.30	5.24 \pm 0.30
Group IV (150 $\mu\text{g}/\text{kg}$ b.w)	12.6 \pm 0.10	37.50 \pm 0.40	6.50 \pm 0.50	5.80 \pm 0.31	5.25 \pm 0.20
Group V (200 $\mu\text{g}/\text{kg}$ b.w)	12.1 \pm 0.30	37.00 \pm 0.30	6.10 \pm 0.30	5.20 \pm 0.40	4.90 \pm 0.40

Values are expressed as mean \pm SD of six animals in each group ($p < 0.05$)

3.3 Antiarthritic effect of silver nanoparticles of *Moringa concanensis* Nimmo

3.3.1 Effect of silver nanoparticles on body weight of FCA induced arthritic rats

The silver nanoparticles treated group of rats (Group IV) showed a similar weight gain compared with control rats

(Group I) but this also showed a significant ($p < 0.05$) increase in the body weight (Table 2) compared with FCA treated rats (Group II). The reference diclofenac treated rats (Group V) also showed a similar weight gain compared with control rats (Group I). This group also showed a significant ($p < 0.05$) increase in the body weight compared to the FCA treated rats (Group II).

Table 2: Effect of AgNPs on body weight of FCA induced arthritic rats

Experimental groups	Initial body weight (g)	Final body weight (g)
Group I (Control)	200 ± 1.05	220 ± 1.10
Group II (FCA)	180 ± 1.10	185 ± 1.30
Group IV (FCA + AgNPs)	210 ± 1.10	217 ± 1.40
A + Diclofenac)	210 ± 1.10	218 ± 1.20

Values are expressed as mean ± S.D for six animals in each group (p<0.05).

3.3.2 Effect of silver nanoparticles on changes in paw volume of FCA induced arthritic rats

The arthritis was induced by the injection of complete freund's adjuvant (FCA) in experimental rats for testing the antiarthritic activity of silver nanoparticles of *Moringa concanensis* Nimmo. The intra peritoneal administration of FCA in the rats (Group II), the results showed the progressive increase in the paw volume compared with control rats (Group I). The difference in the volume of injected

(ipsilateral) and non injected (contralateral) paw between FCA and drug treated rats was statistically significant (p<0.05) in a dose dependent manner (table 3). The paw swelling is the index of measuring the antiarthritic activity of various drugs and employed here to determine the activity of silver nanoparticles of *M.concanensis* Nimmo leaves. The silver nanoparticles administered groups showed a marked reduction in paw volume when compared with the control group.

Table 3: Effect of silver nanoparticles on changes in paw volume (P.V)

Experimental groups	Initial paw volume (ml)	Final paw volume (ml)
Group I (Control)	0.85 ± 0.10	0.86 ± 0.08
Group II (FCA)	0.88 ± 0.06	2.95 ± 0.50
A + AgNPs)	0.86 ± 0.07	1.15 ± 0.10
Group IV (FCA + Diclofenac)	0.85 ± 1.10	1.20 ± 0.20

Values are expressed as mean ± S.D for six animals in each group (p<0.05).

3.3.3 Effect of silver nanoparticles on changes in hematological parameters of FCA induced arthritic rats

The silver nanoparticles of *Moringa concanensis* Nimmo leaves treated group showed remarkable activity in restoration of blood components in arthritis induced rats. The silver nanoparticles of *Moringa concanensis* Nimmo

leaves treated rats (Group III) significantly (p<0.05) increased the hemoglobin, packed cell volume (PCV), red blood cells and platelets in the experimental rats than silver nanoparticles and standard drug diclofenac (Group IV and Group V) compared to the control (Group I). The results showed in (Table 4).

Table 4: Effect of silver nanoparticles on changes in hematological parameters of FCA induced arthritis rats

Experimental groups	Hb (g%)	PCV (%)	WBC (10 ³ /μl)	RBC (10 ¹² /μl)	Platelets (10 ⁹ /μl)	ESR (mm/hr)
Group I (Control)	14.5 ± 0.40	44 ± 0.60	6.60 ± 0.50	6.50 ± 0.40	7.50 ± 0.50	10 ± 0.27
Group II (FCA)	12.0 ± 0.20	36 ± 0.20	11.50 ± 1.45	5.20 ± 0.60	6.30 ± 0.50	22 ± 1.50
Group III FCA + AgNPs)	12.6 ± 0.65	42 ± 0.60	6.30 ± 0.50	6.20 ± 0.70	7.30 ± 0.50	12 ± 0.50
Group V (FCA + Diclofenac)	12.2 ± 0.20	41 ± 0.40	6.10 ± 0.70	6.30 ± 0.70	7.30 ± 0.30	13 ± 0.40

Values are expressed as mean ± S.D for six animals in each group (p<0.05).

3.3.4 Effect of silver nanoparticles on changes in serum liver marker enzymes of FCA induced arthritic rats

In the present study, the challenge with FCA (0.1 ml) significantly (p<0.05) elevated the serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) level. Assessment of the serum

levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) provides an excellent and simple tool to measure the antiarthritic activity of the drug. The FCA induced rats showed a random increase in the liver function marker enzymes and this could lead to the fatality. The silver nanoparticles treated rats showed a notable decrease in the liver marker enzymes (table 5).

Table 5: Effect of silver nanoparticles on changes in serum liver marker enzymes

Experimental groups	AST (μ l)	ALT (μ l)	ALP (μ l)	ACP (μ l)	LDH (μ l)	GGT (μ l)
Group I (Control)	85.23 \pm 1.30	65.94 \pm 0.95	115.39 \pm 1.34	4.9 \pm 0.56	324.72 \pm 1.78	19.34 \pm 1.32
Group II (FCA)	103.01 \pm 1.23	85.00 \pm 1.01	138.65 \pm 1.55	5.3 \pm 0.60	432.45 \pm 2.10	31.36 \pm 1.26
Group III (FCA + AgNPs)	87.13 \pm 1.35	67.43 \pm 0.93	116.02 \pm 1.31	5.0 \pm 0.61	358.12 \pm 1.46	22.98 \pm 1.10
Group V (FCA + Diclofenac)	87.26 \pm 1.29	69.01 \pm 0.87	116.52 \pm 1.22	5.1 \pm 0.57	363.00 \pm 1.48	22.26 \pm 1.37

Values are expressed as mean \pm S.D for six animals in each group ($p < 0.05$)

3.3.5 Effect of AgNPs on changes in serum glucose of FCA induced arthritic rats

The serum glucose level of silver nanoparticles treated experimental rats (Group IV) showed a significant ($p < 0.05$) decrease when compared to the FCA treated experimental rats (Group II). The silver nanoparticles administered rats showed a significantly ($p < 0.05$) slight increase when compared to control rats (Group I) and the EEMC treated (Group III) rats.

Table 6: Effect of AgNPs on changes in serum glucose of FCA induced arthritic rats

Experimental Groups	Glucose (mg/dl)
Group I (Control)	106.25 \pm 0.73
Group II (FCA)	140.22 \pm 0.67
Group III (FCA + AgNPs)	108.12 \pm 0.63
Group IV (FCA + Diclofenac)	109.28 \pm 0.54

Values are expressed as mean \pm S.D for six animals in each group ($p < 0.05$)

The silver nanoparticles treated rats also showed a significant ($p < 0.05$) decrease in the serum glucose level of experimental rats when compared to standard drug diclofenac treated (Group V) experimental rats (Table 6).

3.3.6 Effect of silver nanoparticles on changes in serum cholesterol and triglycerides of FCA induced arthritic rats

The serum cholesterol level was rapidly increased after the induction of arthritis by using complete Freund's adjuvant (Group II). The silver nanoparticles showed a significant ($p < 0.05$) decrease in the serum cholesterol level of experimental rats (Group III) when compared to FCA treated rats (Group II). The serum triglycerides level was dramatically increased after the induction of arthritis by using complete Freund's adjuvant (Group II) (table 7). The silver nanoparticles showed a significant ($p < 0.05$) decrease in the serum triglycerides level of experimental rats (Group III) when compared to the FCA treated rats (Group II).

Table 7: Effect of silver nanoparticles on changes in serum cholesterol and triglycerides

Experimental groups	Total cholesterol (mg/dl)	Total triglycerides (mg/dl)
Group I (Control)	73.45 \pm 1.23	63.20 \pm 0.55
Group II (FCA)	96.32 \pm 0.21	88.45 \pm 0.95
Group III (FCA + AgNPs)	76.48 \pm 0.92	67.34 \pm 0.40
Group IV (FCA + Diclofenac)	77.37 \pm 0.27	68.38 \pm 0.28

Values are expressed as mean \pm S.D for six animals in each group ($p < 0.05$)

3.3.7 Effect of silver nanoparticles on changes in serum of renal function markers of FCA induced arthritic rats

The estimation of renal function markers in the serum is an important parameter for assaying the antiarthritic activity of plant extracts and drugs. The FCA treated rats showed a

dramatically increased level in the renal function markers such as, urea, creatinine, uric acid and albumin. The treatment with silver nanoparticles of *M.concanensis* leaves showed a significant ($p < 0.05$) restoration of renal function markers (table 8).

Table 8: Effect of silver nanoparticles on changes in serum of renal function markers of FCA induced arthritic rats

Experimental groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Protein g/dl)	Albumin (g/dl)	CRP (μ g/ml)
Group I (Control)	14.07 \pm 0.76	0.76 \pm 0.05	2.05 \pm 0.15	6.35 \pm 0.45	3.71 \pm 0.25	4.64 \pm 0.59
Group II (FCA)	26.2 \pm 0.95	2.02 \pm 1.05	3.21 \pm 0.23	4.25 \pm 0.83	2.45 \pm 0.22	20.53 \pm 1.67
Group III (FCA + AgNPs)	15.47 \pm 0.65	0.97 \pm 0.06	2.22 \pm 0.10	6.30 \pm 0.47	3.23 \pm 0.28	8.28 \pm 0.27
Group V (FCA + Diclofenac)	16.83 \pm 0.66	1.00 \pm 0.09	2.53 \pm 0.14	6.25 \pm 0.50	3.45 \pm 0.31	9.77 \pm 0.42

Values are expressed as mean \pm S.D for six animals in each group ($p < 0.05$)

3.4 Histopathological evaluation of hind limb

Histopathological assessment of joints from treated and control rats revealed remarkable signs of cellular infiltration, synovial hyperplasia, pannus formation, partial cartilage and bone destruction in the untreated FCA rats.

The normal connective tissue structure with the absence of necrosis in the tibiotarsal joint was observed in the control group. The silver nanoparticles treated rats produced knee joint protection compared to arthritic rats by reducing the inflammation and necrosis. However, the synthesized silver

nanoparticles treated rats had significantly ($p < 0.05$) decreased the levels of cellular infiltration, hyperplasia and bone destruction and this improved histopathology

compared to the FCA treated rats (Figure 1). Rats treated silver nanoparticles showed mild necrosis with edema but granuloma was absent in tibiotarsal joint.

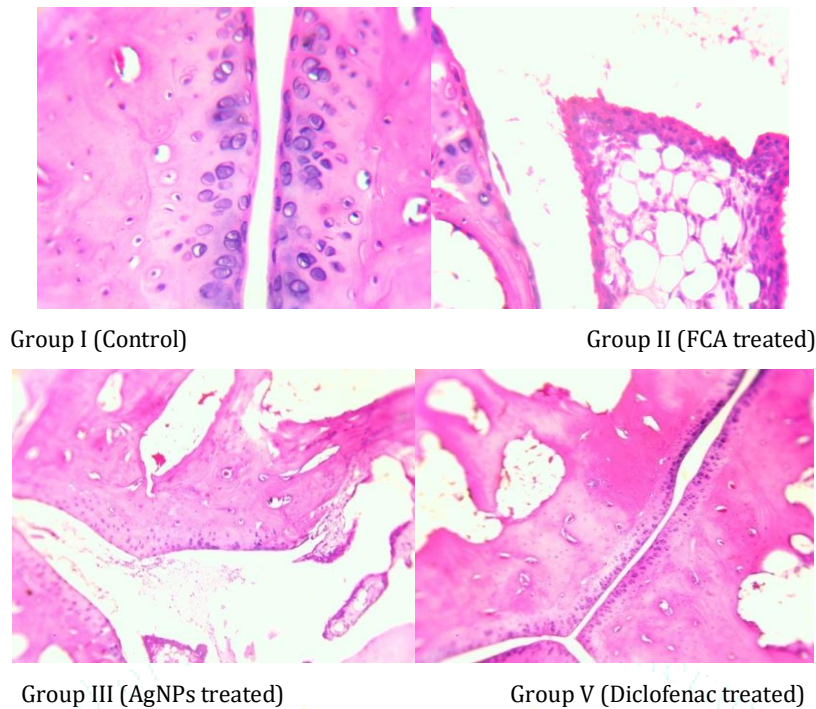


Figure 1: Histopathological analysis of hind limb

3.5 Histopathological analysis of liver

Histological changes in the liver there was a no toxic effect on the liver of experimental rats with FCA treated was detected after the treatment with silver nanoparticles. The AgNPs treatment decreased the alteration of hepatic

parenchyma and general inflammatory infiltration of hepatic stroma in comparison with the control group. Slight observed dystrophy in the liver of the silver nanoparticles treated groups means the alterations some of which maybe are reversible while a hard dystrophy can be irreversible, that means progressing to necrosis.

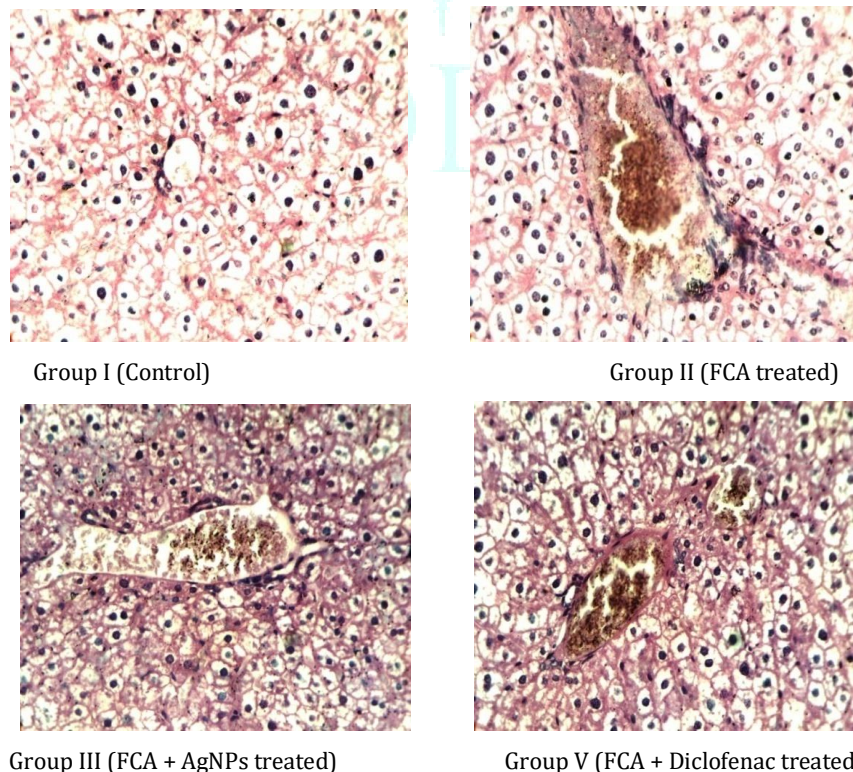


Figure 2: Histopathological analysis of liver

4. DISCUSSION

Rheumatoid arthritis is a long term inflammatory autoimmune disease that causes chronic joint inflammation with the symptoms of pain, swelling, and stiffness in the hands, feet, knees, and wrists. Rheumatoid arthritis is generally characterized by chronic synovial inflammation of the joints, resulting in the degradation and destruction of cartilages and bones, and has poor outcomes with limited treatment options. Despite the lack of robust epidemiological studies in some regions and the difference in Rheumatoid arthritis prevalence between ethnicities, the prevalence of Rheumatoid arthritis is the highest among the inflammatory autoimmune diseases, ranging from 0.5 to 1% of the population worldwide¹³⁻¹⁶. Freund's complete adjuvant induced arthritis in rat model is the best and most widely used experimental model for arthritis. It is a T cell and neutrophil dependent and complement independent helper inflammatory cytokines are associated CFA induced arthritis¹⁷.

Silver was the only material that the plasmon resonance can be turned to any wavelength in the visible spectrum⁸. Studies carried out in the last few decades shows that silver nanoparticles exhibit a rare combination of valuable properties like catalytic activity, high electrical double layer capacitance etc. Nanosilver has also been used extensively as antibacterial agent in the health industry, food storage, textile and a number of environmental applications¹⁸⁻²⁰. The rheumatoid arthritis is associated with the weight loss and this loss of body weight leads to the lean body, known as rheumatoid cachexia. Rheumatoid cachexia is thought to be the end result of cytokine driven hyper metabolism and was a key comorbidity in rheumatoid arthritis^{21,22}.

Reduction in bone configuration and increased bone resorption are the causes of bone loss in FCA induced arthritic rats. This has been reported that a moderate rise in the WBC count occurs in arthritic conditions due to an IL-1 β mediated rise in the respective colony stimulating factors and reduction in hemoglobin count in arthritis results from reduced erythropoietin levels, a decreased response of the bone marrow erythropoietin and premature destruction of red blood cells²³. The experimental rats treated with FCA exhibited the rapid elevation in white blood cells (WBC) and erythrocytes sedimentation rate (ESR) and exhibited the decreases in red blood cells (RBC), platelets and hemoglobin. The AgNPs showed the noticeable elevation in red blood cells, hemoglobin and platelets and also prevent the rapid elevation of white blood cells and erythrocyte sedimentation rate. In these results a slight differences was noted, that the AgNPs possess the better activity.

The rats treated with the FCA showed the rapid increases in the liver marker enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and gamma glutamyl transferase (γ -GT). The increases of these enzymes may possess the deformity of liver function. The AgNPs exhibited the marked decreases and set back to control of these enzymes in experimental rats. The serum glucose, cholesterol, triglycerides and urea level was rapidly increased in the rats after the FCA treatment. The AgNPs was exhibited the decrease of glucose, cholesterol, triglycerides, creatinine, uric acid and urea level in the serum of experimental rats.

Histopathological analysis of joints of FCA treated and control rats revealed remarkable signs of cellular infiltration, synovial hyperplasia, pannus formation, partial cartilage and bone destruction in the FCA treated rats compared to control. The AgNPs treated rats produced knee joint

protection compared to arthritic rats by reducing the inflammation and necrosis. However, AgNPs treated rats were decreased the levels of cellular infiltration, hyperplasia and bone destruction and this improved histopathology was contributed by the silver nanoparticles. The rats treated silver nanoparticles showed mild necrosis with edema but granuloma was absent in tibiotarsal joint. Histological changes in the liver there was a no toxic effect on the liver of experimental rats with FCA treated was detected after the treatment with AgNPs. Both treatments decreased the alteration of hepatic parenchyma and general inflammatory infiltration of hepatic stoma in comparison with the control group. Based on the present study, this was clear that the new finding clearly indicates that the silver nanoparticles from *Moringa concanensis* Nimmo leaves was possessed the excellent antiarthritic activity.

5. CONCLUSION

The present study was concluded with the new findings was clearly indicates that the silver nanoparticles from *Moringa concanensis* Nimmo leaves was possessed the notable antiarthritic activity. These results may leads to the development of novel antiarthritic drugs from the plant *Moringa concanensis* Nimmo.

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