

Research Article**Open Access****Assessment of antiarthritic activity of ethanolic extract of *Moringa concanensis* Nimmo leaves by using FCA induced arthritic animal model**

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Abstract: The present research work was dealing with the evaluation of the antiarthritic potential of *M.concanensis* leaves by using FCA induced arthritic animal model. The arthritis was induced by complete Freund's adjuvant method and treated with ethanolic extract of *M.concanensis* leaves (EEMC). The body weight, paw volume, hematological parameters, liver function marker enzymes and the renal function markers of the arthritic rats and EEMC rats was analyzed by the standard methods. The new findings from the present study the ethanolic extract of *M.concanensis* leaves was showed a potent antiarthritic activity against FCA induced rheumatoid arthritis in experimental rats. Based on the above findings, it was concluded as this research work may give a clear understanding on the antiarthritic activity of ethanolic extract of the leaves of *Moringa concanensis* Nimmo. This result may be used in the further research and new findings in this plant.

Key words: *M.concanensis* leaves, arthritis, FCA, diclofenac and Wistar rats.

Introduction

Rheumatoid arthritis is an autoimmune disease with joint inflammation, synovial proliferation and destruction of articular cartilage and the majority is seen in females. Prolonged treatment with allopathic drugs causes adverse side effects and hence alternative natural measures are in search. The production of auto antigens in certain arthritic diseases may be due to *in vivo* denaturation of proteins and the effect can be reduced by inhibiting protein denaturation and regulating the production of auto antigen (Khader *et al.*, 2018).

Inflammation is one of the major causes of the development of various diseases like arthritis, cancer, cardiovascular disease, diabetes, obesity, osteoporosis, inflammatory bowel disease, asthma, and even central nervous system (CNS)-related diseases such as depression and Parkinson's disease (Laveti *et al.*, 2013). Reports in the literature suggest that almost 90% of synthetic anti-inflammatory drugs produce drug-related toxicities, idiosyncratic reactions, and adverse effects compromising the treatment process (Lanas, 2009; Kandulski *et al.*, 2009). Consequently, an immense interest has reemerged in herbs exerting anti-inflammatory activity as potential alternative or concomitant tools for anti-inflammatory treatment

with much less side effects. The present study deals with the assessment of antiarthritic activity of ethanolic extract of *M.concanensis* leaves in experimental rats.

Material and Methods**Collection and identification of plant**

The healthy, matured and insect bites free leaves of *Moringa concanensis* Nimmo plant (Family - *Moringaceae*) was collected (Figure 1) from Esanai village, Perambalur district, Tamilnadu (Figure 2), 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.

Preparation of plant extracts

The *Moringa concanensis* Nimmo leaves were washed, shade dried and powdered using a mixer grinder. The powdered leaves (10 gms) were extracted with 100 ml of selected organic solvent i.e., ethanol by using Soxhlet apparatus. The concentrated solvent extract of the leaves was stored in refrigerator for further analysis.

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Preliminary animal studies in toxicity studies of dose fixing for ethanolic extract of *Moringa concanensis* Nimmo leaves

Experimental design

Group I: Normal rats (Standard diet)

Group II: Rats fed orally with ethanolic extracts of *M.concanensis* Nimmo leaves of 100 mg/kg body weight for 14 days.

Group III: Rats fed orally with ethanolic extracts of *M.concanensis* Nimmo leaves of 200 mg/kg body weight for 14 days.

Group IV: Rats fed orally with ethanolic extracts of *M.concanensis* Nimmo leaves of 400 mg/kg body weight for 14 days.

Group V: Rats fed orally with ethanolic extracts of *M.concanensis* Nimmo leaves of 800 mg/kg body weight for 14 days.

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 paw (Mi-Jung et al., 2006).

Experimental design

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 ethanolic extract of *M.concanensis* Nimmo leaves (200 mg / kg body weight) by oral administration for 28 days.

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

Collection of samples

After 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.

Body weight

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

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).

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 - 800i automatic haematology analyzer.

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.

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 analyzed for the changes in the blood cells using hematology analyzer.

Estimation of liver marker enzymes

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.

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.

Estimation of alkaline phosphatase

Pipette 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.

Estimation of acid phosphatase

Pipette 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 2ml of sodium carbonate. Take 4 ml of working standard solution and for blank taken 3.2ml water and 0.8 ml of phenol reagent. Then add 2ml 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.

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.

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.

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.

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.

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

2ml 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.

Estimation of renal function markers

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 diacetylmonoxime 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 480nm in a UV-visible spectrophotometer. Aliquots of urea were also treated in a similar manner. The value was expressed as mg/dl blood.

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 0.75N 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.

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.

Estimation of protein

Pipette 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 mins, the blue colour developed was measured at 660 nm.

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 (620–650nm). The values were expressed in g / dl in serum.

Estimation of C - Reactive protein (CRP)

Pipette out the 990 µl of wash buffer into a test tube. Add 10 µl of the provided standard (1:100 dilutions) to create the top standard, 10 ng / ml. Dilute the 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 the 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. 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.

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.

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 - 60°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.

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 60°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.

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.

Results

Effect of ethanolic extract of *Moringa concanensis* leaves on hematological parameters in blood of experimental rats

The dose fixation study was employed in various concentration of EEMC (100 mg/kg b.wt to 600 mg/kg b.wt). The obtained result (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 200 mg/ kg of body weight in very close relation with control group. However, 400 mg/ kg of body weight and 600 mg/ kg of body weight of EEMC 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 ethanolic extracts of *Moringa concanensis* Nimmo leaves (table 1).

Table 1: Effect of ethanolic extract of *Moringa concanensis* Nimmo leaves 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 (100 mg / kg b.w)	12.6 \pm 0.50	37.50 \pm 1.10	6.20 \pm 0.30	5.70 \pm 0.50	5.20 \pm 0.40
Group III (200 mg / kg b.w)	12.6 \pm 0.10	37.50 \pm 0.50	6.40 \pm 0.40	5.80 \pm 0.50	5.25 \pm 0.30
Group IV (400 mg / kg b.w)	12.1 \pm 0.15	36.12 \pm 0.60	5.23 \pm 0.10	5.10 \pm 0.50	5.12 \pm 0.30
Group V (800 mg / kg b.w)	11.8 \pm 0.20	36.05 \pm 1.20	5.08 \pm 0.30	5.15 \pm 0.40	4.96 \pm 0.20

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

Here the present study revealed that the, 200 mg/ kg of dose did not showed any harmful effects in treated rats but there was a significant ($p < 0.05$) decrease in the blood components of experimental rats treated with EEMC 400 mg/ kg of body weight and 600 mg/ kg of body weight as compared with control rats. This result clearly indicates that the

200 mg/ kg per body weight was the optimum dose for this investigation.

Antiarthritic effect of ethanolic extract of *Moringa concanensis* Nimmo

Effect of ethanolic extract of *Moringa concanensis* leaves on body weight of FCA induced arthritic rats

The ethanolic extract of *Moringa concanensis* Nimmo leaves treated rats showed remarkable activity (table 2). The EEMC treated group of rats (Group III) showed a significant ($p < 0.05$) increases in the body weight compared with control group (Group I). The EEMC treated rats showed a significant ($p < 0.05$) increases in the body weight compared with FCA treated rats (Group II) and the reference drug diclofenac treated group (Group IV).

Table 2: Effect of *Moringa concanensis* Nimmo leaves 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 III (FCA + EEMC)	210 ± 1.05	221 ± 1.10
Group IV (FCA + Diclofenac)	210 ± 1.10	218 ± 1.20

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

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). The significant ($p < 0.05$) increases in the weight was observed upon physical assessment of arthritic rats treated with EEMC. Therefore, this result indicates that the plant *Moringa concanensis* Nimmo was source for good management of rheumatoid cachexia and minimizing the risk of mortality in RA.

Effect of ethanolic extract of *Moringa concanensis* Nimmo leaves and 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 ethanolic

leaves extract 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 ethanolic extract of *Moringa concanensis* Nimmo leaves treated rats (Group III) showed the significant ($p < 0.05$) decreases in the paw volume compared to the FCA induced rats (Group II). These results were clearly indicates the ethanolic extract of *M. concanensis* Nimmo leaves and silver nanoparticles was significantly ($p < 0.05$) decreased the paw volume of FCA induced experimental rats (table 3).

Table 3: Effect of ethanolic extract of *Moringa concanensis* Nimmo leaves 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
Group III (FCA + EEMC)	0.90 ± 0.04	1.10 ± 0.40
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 ($p < 0.05$).

Effect of ethanolic extract of *Moringa concanensis* leaves on changes in hematological parameters of FCA induced arthritic rats

The ethanolic extract of *Moringa concanensis* Nimmo leaves showed remarkable activity in restoration of blood components in arthritis induced rats. The ethanolic extract 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 standard drug diclofenac (Group IV) compared to the control (Group I). The results showed in (Table 4).

Table 4: Effect of *Moringa concanensis* Nimmo leaves on changes in hematological parameters of FCA induced arthritis rats

Experimental groups	Hb (g%)	PCV (%)	WBC ($10^3/\mu\text{l}$)	RBC ($10^{12}/\mu\text{l}$)	Platelets ($10^9/\mu\text{l}$)	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 + EEMC)	13.5 ± 0.30	43 ± 0.30	6.80 ± 1.40	6.40 ± 0.80	7.40 ± 0.60	11 ± 0.80
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 ($p < 0.05$).

The present study revealed that *Moringa concanensis* Nimmo and diclofenac treatments significantly decreased ($p < 0.05$) the WBC count

and increased the Hb level. In addition to this, other characteristic hematological alterations such as the decreased RBC and increased platelet count

were also significantly restored by the *Moringa concanensis* Nimmo and synthesized silver nanoparticles. Also a persistent high serum level of C-reactive protein (CRP) was recognized as strong indicator of rheumatoid arthritis.

Effect of ethanolic extract of *Moringa concanensis* leaves 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 (table 5). The FCA induced rats showed a random increase in the liver function marker enzymes and this could lead to the fatality. The AgNPs treated rats showed a notable decrease in the liver marker enzymes.

Table 5: Effect of *Moringa concanensis* Nimmo leaves on changes in serum liver marker enzymes

Experimental groups	AST (µl)	ALT (µl)	ALP (µl)	ACP (µl)	LDH (µl)	GGT (µl)
Group I (Control)	85.23 ±1.30	65.94±0.95	115.39±1.34	4.9±0.56	324.72±1.78	19.34±1.32
Group II (FCA)	103.01±1.23	85.00±1.01	138.65±1.55	5.3±0.60	432.45±2.10	31.36±1.26
Group III (FCA + EEMC)	86.20±1.20	66.31±0.98	115.11±1.45	4.9±0.63	325.45±1.67	20.26±1.15
Group V (FCA + Diclofenac)	87.26±1.29	69.01±0.87	116.52±1.22	5.1±0.57	363.00±1.48	22.26±1.37

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

Effect of ethanolic extract of *Moringa concanensis* Nimmo leaves on changes in serum glucose of FCA induced arthritic rats

The serum glucose level was dramatically increased after the induction of arthritis by using complete Freund's adjuvant (Group II). The ethanolic extract of *Moringa concanensis* Nimmo leaves showed a significant ($p < 0.05$) decrease in the serum glucose level of experimental rats (Group III) when compared to the FCA treated rats (Group II). The ethanolic extract of *Moringa concanensis* Nimmo leaves showed a significantly ($p < 0.05$) slight increase in the serum glucose level when compared to the control group rats (Group I) (table 6). The treatment with ethanolic extract of *Moringa concanensis* Nimmo leaves showed a significant ($p < 0.05$) decrease when compared to silver nanoparticles treated rats (Group IV) and the standard drug diclofenac treated rats (Group V).

ethanolic extract of *Moringa concanensis* Nimmo leaves showed a significant ($p < 0.05$) decrease in the serum cholesterol level of experimental rats when compared to the standard drug diclofenac treated experimental rats (Group IV).

Table 6: Effect of *Moringa concanensis* Nimmo leaves on changes in serum glucose of FCA induced arthritic rats

Experimental Groups	Glucose (mg/dl)
Group I (Control)	106.25 ± 0.73
Group II (FCA)	140.22 ± 0.67
Group III (FCA + EEMC)	107.28 ± 0.43
Group V (FCA + Diclofenac)	109.28 ± 0.54

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

Effect of ethanolic extract of *Moringa concanensis* Nimmo leaves 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 ethanolic extract of *Moringa concanensis* Nimmo leaves 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 ethanolic extract of *Moringa concanensis* Nimmo leaves showed a significantly ($p < 0.05$) slight increase in the serum cholesterol level compared to control rats (Group I) (table 7). The treatment with

Table 7: Effect of *Moringa concanensis* Nimmo leaves on changes in serum cholesterol and triglycerides

Experimental groups	Total cholesterol (mg/dl)	Total triglycerides (mg/dl)
Group I (Control)	73.45 ± 1.23	63.20 ± 0.55
Group II (FCA)	96.32 ± 0.21	88.45 ± 0.95
Group III (FCA + EEMC)	74.40 ± 1.25	65.27 ± 0.39
Group V (FCA + Diclofenac)	77.37 ± 0.27	68.38 ± 0.28

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

Effect of ethanolic extract of *Moringa concanensis* leaves 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 urea level in the serum of experimental rats

was dramatically increased after the induction of arthritis by using complete Freund's adjuvant (Group II). The ethanolic extract of *Moringa concanensis* Nimmo leaves showed a significantly ($p < 0.05$) decreased in the urea level of experimental rats (Group III) when compared to the FCA treated rats (Group II).

The total protein of the experimental rats decreased abnormally after the FCA induction of arthritis. The ethanolic extract of *Moringa concanensis* Nimmo leaves showed a significant ($p < 0.05$) increases in the total protein level in the serum of experimental rats when compared to the FCA treated (Group II) experimental rats (table 8). The treatment with the EEMC showed a significant ($p < 0.05$) increase in the total protein content in the serum of experimental rats compared to the control rats (Group I).

Histopathological evaluation of hind limb

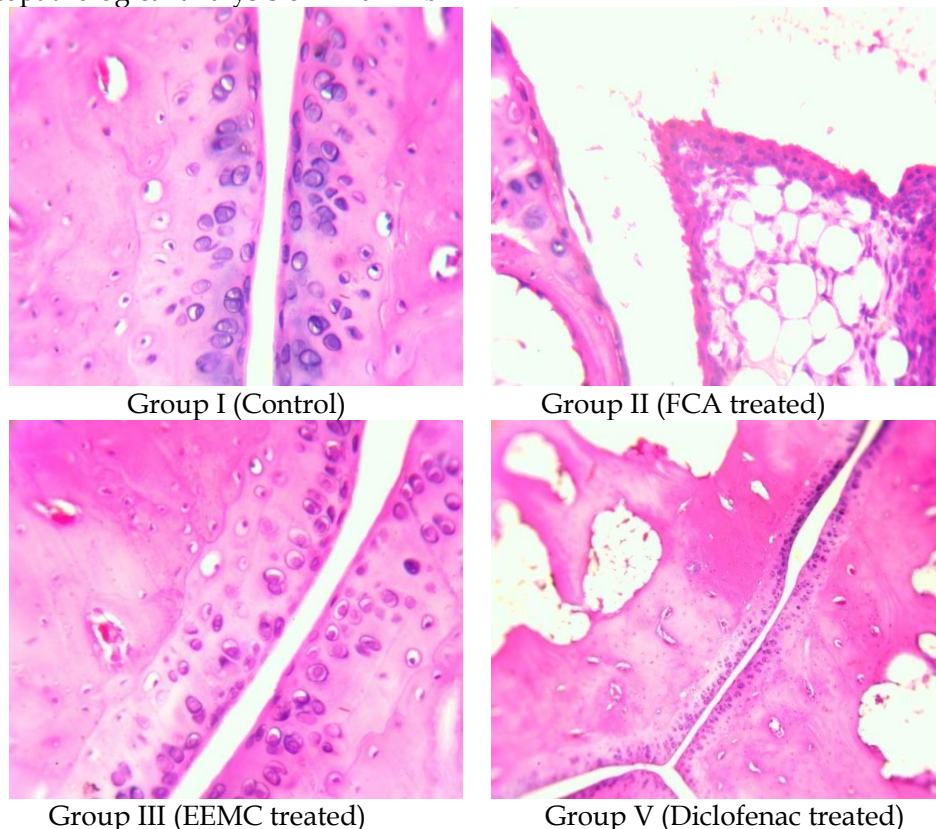
Histopathological assessment of joints from treated and control rats revealed remarkable signs of cellular infiltration, synovial hyperplasia, pannus formation, partial cartilage and bone destruction in the untreated FCA rats. The normal connective tissue structure with the absence of necrosis in the tibiotarsal joint was observed in the control group. The EEMC treated rats produced knee joint protection compared to arthritic rats by reducing the inflammation and necrosis. However, the EEMC treated rats had significantly ($p < 0.05$) decreased the levels of cellular infiltration, hyperplasia and bone destruction and this improved histopathology was contributed by the EEMC compared to the FCA treated rats (Figure 1).

Table 8: Effect of *Moringa concanensis* Nimmo leaves 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 (µg/ml)
Group I (Control)	14.07 ± 0.76	0.76 ± 0.05	2.05 ± 0.15	6.35 ± 0.45	3.71 ± 0.25	4.64 ± 0.59
Group II (FCA)	26.2 ± 0.95	2.02 ± 1.05	3.21 ± 0.23	4.25 ± 0.83	2.45 ± 0.22	20.53 ± 1.67
Group III (FCA + EEMC)	15.32 ± 0.75	0.78 ± 0.07	1.94 ± 0.12	6.97 ± 0.34	3.55 ± 0.25	6.36 ± 0.34
Group V (FCA + Diclofenac)	16.83 ± 0.66	1.00 ± 0.09	2.53 ± 0.14	6.25 ± 0.50	3.45 ± 0.31	9.77 ± 0.42

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

Figure 1: Histopathological analysis of hind limb

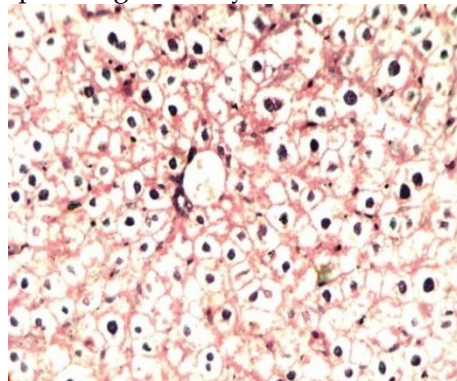


Histopathological analysis of liver

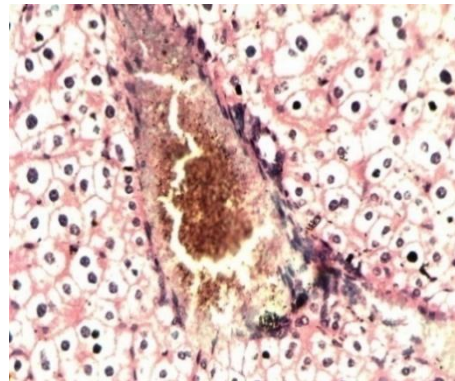
Histological changes in the liver there was a no toxic effect on the liver of experimental rats with FCA treated was detected after the treatment with ethanolic extract of *Moringa concanensis* Nimmo leaves (Figure 2). The EEMC decreased the alteration of hepatic

parenchyma and general inflammatory infiltration of hepatic stroma in comparison with the control group. The EEMC significantly ($p < 0.05$) diminished the inflammatory infiltration of hepatic stroma with granulocytes when compared to the control group of rats (Figure 2).

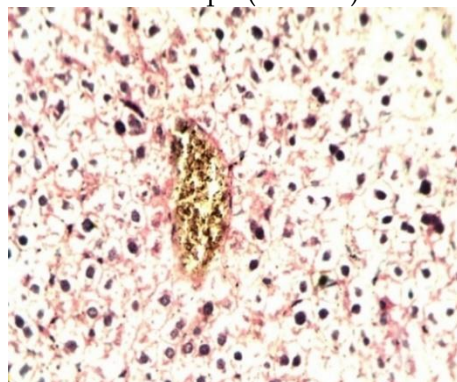
Figure 2: Histopathological analysis of liver



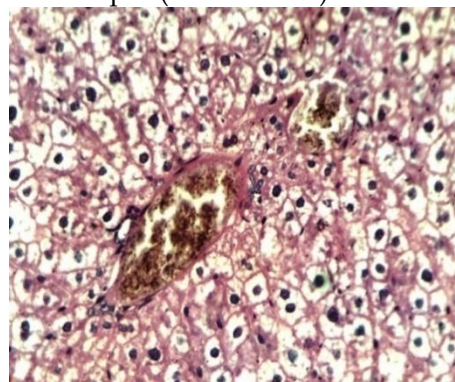
Group I (Control)



Group II (FCA treated)



Group III (FCA + EEMC treated)



Group V (FCA + Diclofenac treated)

Discussion

Inflammation is an innate immune mechanism to protect the body from invading pathogens and cellular danger signals (Janeway and Medzhitov, 2002; Yi, 2016; Yi, 2017). Although an inflammatory response is a host-defense mechanism, chronic inflammation, defined as repeated and prolonged inflammation, is considered a major risk factor for the development of inflammatory and autoimmune diseases. Rheumatic diseases are chronic inflammatory and degenerative autoimmune diseases that primarily affect connective tissues such as cartilage and bones, ligaments, tendons, and muscles but can also affect nonconnective tissues and internal organs, leading to substantial morbidity (Yi, 2018).

More than 100 rheumatic diseases have been identified, and there are numerous patients who suffer from these diseases worldwide. This has

resulted in extensive investigation of the mechanisms of disease pathogenesis and development of effective therapeutics. Representative rheumatic diseases include rheumatoid arthritis (RA), osteoarthritis (OA), systemic lupus erythematosus (SLE), ankylosing spondylitis, and Sjogren's syndrome. Despite uncertainty regarding the etiology of rheumatic diseases, chronic inflammation and autoimmunity are known to play critical roles in both the onset and progression of rheumatic diseases.

In the present study, the ethanolic extract of *M. concanensis* leaves (EEMC) was evaluated against the FCA induced rheumatoid arthritis in experimental rats. In rats, body weight was directly proportional to food intake and metabolism, but this might be affected by immunity and inflammation, regulated by the hormone leptin

(Pankaj et al., 2015). Progression of disease status and response to anti-inflammatory therapy are indirectly linked with change in body weight (Patil et al., 2011). The loss of lean body mass is associated with decreased physical activity, muscle strength, and endurance in performing activities of daily living (Rall and Roubenoff, 1996). A loss greater than 40% of existing lean body mass resulted in RA often leads to death (Roubenoff and Rall, 1993). The measurement of paw or joint swelling gives an indication of edematous changes in this region, but the actual damage takes place in the tibiotarsal joint (Escandell et al., 2007). Reduction in bone configuration and increased bone resorption are the causes of bone loss in FCA induced arthritic rats (Aota et al., 1996; Findlay and Haynes 2005; Makinen et al., 2007). 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 cells (Jalalpure et al., 2011). White blood cells are a major component of the body's immune system. Indications for a WBCs count include infections and inflammatory disease (Sharma and Kumar, 2009). Lymphocytes play a central role in the pathogenesis of RA. The rheumatoid synovium contains large numbers of lymphocytes and plasma cells, many of which bear the markers of activation (Symmons et al., 1989).

Reasons that could cause reduction in hemoglobin level in RA include a blunted erythropoietin response by the RBC precursors, decreased survival of the RBCs, defective iron absorption and glucocorticoids influence (Kulich et al., 2002). The ankle joint of disease controlled arthritic rat, showed prominent abnormalities like edema formation, degeneration with erosion of the cartilage, and extensive infiltration of inflammatory exudates in the articular surface (Nipate et al., 2015). Numerous studies have shown that in the course of arthritis not only joints with obvious signs of inflammation, but also visceral organs may be affected by the pathological process and the weight of internal organs, such as liver and spleen increase (Fedatto Junior et al., 1999; Hemshekhar et al., 2010). Based on the above findings ethanolic extract of *M.concanensis* leaves


(EEMC) was showed a acceptable antiarthritic activity in FCA induced arthritis in rats.

Conclusion

Based on the present study, this was concluded that the new finding clearly indicates that the plant *Moringa concanensis* Nimmo leaves was possessed the excellent antiarthritic activity. These results may leads to the development of novel antiarthritic drugs from the plant *Moringa concanensis* Nimmo and also to improve the health status of the consumers as a result of the presence of various components that are vital role for good health.

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