World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Evaluation of anti-arthritic potential of NONI extract in experimental rats

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Received: 24-12-2014 / Revised: 21-01-2015 / Accepted: 26-01-2015

ABSTRACT

Morinda citrifolia popularly known as Noni, is well known for its medicinal properties in folk medicine. The methanolic fruit extract of Noni was evaluated for anti-arthritic activity in rats by Complete Freund's adjuvant method using Glucosamine sulphate (300 mg/kg) as the standard drug. The parameters such as paw volume, body weight, copper level and hematological parameters was determined and histopathological studies was carried out to assess the effect of extract on joints of the hind paw. Immunological parameters were estimated to determine the level of C-reactive protein. Results showed significant anti-arthritic activity of Noni extract in experimental rat model.

Key words: Anti-arthritic, Complete Freund's adjuvant, *Morinda citrifolia*, Noni.

INTRODUCTION

Rheumatoid arthritis (RA), one of the commonest autoimmune diseases, is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction, which is responsible for the deformity and disability.[1] Rheumatoid arthritis has a worldwide distribution with an estimated prevalence of 1 to 2%. Prevalence increases with age, approaching 5% in women over age 55. The average annual incidence in the United States is about 70 per 100,000, annually.[2] Herbal and natural products of folk medicine have been used for centuries for the treatment of various disorders throughout the world. *Morinda citrifolia* L (Rubiaceae), known as Noni, is widely distributed in tropical Asia, India, and the Pacific Islands. Almost all parts of this plant including fruits, flowers, leaves, bark, stem, and roots have been used as food, medicine, and fabric dyes for more than 2000 years by the Polynesian people. The plant has displayed antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory, and immune enhancing activities in pharmacological studies.[3] Based on the ethnomedical uses of the plant an attempt was made to study the anti-arthritic activity of Noni fruit extract in experimental rats by Complete Freund's adjuvant method.

MATERIALS AND METHODS

Plant Material and preparation of extract: Fruits of *Morinda citrifolia* was collected from J.K exports, Salem and authenticated (Authentication Ref. no: DSCP/Auth/2319) by a botanist. The powdered material of the fruits was refluxed successively with the solvents, Petroleum ether and methanol in a Soxhlet extractor for 48 hrs in batches of 500 g each. Both extracts so obtained were concentrated in vacuum using rotary flash evaporator and dried in dessicators.[4] The extracts so obtained were labeled, weighed and the yield was calculated in terms of grams percent of the weight of the powdered fruits.

Experimental Animals: Healthy Wistar rats and Swiss Albino mice of either sex, weighing between 150-200 g and 20-30 g respectively were procured from the animal house of Dayananda Sagar college of Pharmacy, Bangalore, India. The animals were lodged in large and spacious hygienically maintained cages during the course of the experimental period. The room temperature was maintained at 25 ± 1°C. The animals were fed with standard rat feed and water ad libitum. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (approval no. Col/IAEC/86/12-13)

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Acute toxicity studies: In the present study the methanolic extract of *Morinda citrifolia* fruits (MEMC) was subjected to acute oral toxicity studies according to OECD guideline No. 425.[5]

Experimental design for complete Freund's adjuvant induced arthritic model: Arthritis was induced by injecting 0.1 ml of Complete Freund's adjuvant into the sub planter region of the left hind paw of male albino rats on day one of the experiment.[6] This consists of 6 mg *mycobacterium butyricum* being suspended in heavy paraffin oil to give 6 mg/ml.Animals were randomly divided into five groups of six animals each and treated for 14 days. Dosing with the test compound or the standard was started on 14th day and continued for 14 days. Group 1 served as normal control and received only distilled water. Group 2 served as arthritic control and was treated only with the vehicle. Group 3 and 4 received 250 mg/kg and 500 mg/kg b.w of extract respectively and group 5 received the standard drug, Glucosamine sulphate at a dose of 300 mg/kg b.w orally. Paw volume was measured in which the displacement volume was measured when the left hind paw is dipped in a plethysmometer. Body weight was measured by digital weighing machine from time to time.[7] C-reactive protein is a member of the class of acute phase reactants as its level rises dramatically during inflammatory processes. CRP level was measured using immunoturbidimetry method.[8]

Ceruloplasmin is an enzyme synthesized in the liver containing eight atoms of copper in its structure. Free Cu ions are powerful catalysts of free radical damage. By binding Cu, ceruloplasmin prevents free Cu ions from catalyzing oxidative damage. The increased level of Cu ion indicates the inflammatory condition. The Cu levels were estimated using UV visibility spectrophotometer by calibration curve method. Complete Blood Count was measured in automated blood count machine. Histopathology studies were carried out to examine for Cartilage destruction and swelling. The section was stained with Haematoxylin and Eosin and observed under the microscope with magnification of 50 X and 400 X.[9]

RESULTS

Acute toxicity studies and selection of dose: Animals treated with 5000 mg/kg b.w of methanolic extract of *Morinda citrifolia* fruits did not produce any mortality after 24 h and upto 14 days. Thus 1/10th and 1/20th of maximum tolerated dose of 5000 mg/kg body weight (250 mg/kg and 500 mg/kg) was selected for evaluation of anti-arthritic activity in rats.

Anti-arthritic activity of MEMC on Complete Freund's adjuvant (CFA) induced RA model:

Effect of MEMC on paw volume: The paw volume of different group of animals during the treatment period has been represented in Table 1. The paw volume of the arthritis control group was found to be 1.5 ± 0.068 on the 28th day. MEMC at a dose of 250 mg/kg and 500 mg/kg caused a dose dependant reduction in paw volume which was found to be 1.36 ± 0.033 and 1.33 ± 0.033 respectively which were comparable to the standard used. The standard group showed a paw volume of 1.25 ± 0.061.

Effect of MEMC on body weight: The body weight of different group of animals during the treatment period has been represented in Table 2. The body weight of the arthritic control group was found to be reduced gradually from 207.66 ± 7.54 to 194 ± 8.04. MEMC at a dose of 250 mg/kg and 500 mg/kg caused decrease in body weight initially from 233.33 ± 7.53 to 211.3 ± 8.71 and 204.66 ± 8.92 to 185.16 ± 12.79 and showed a dose dependent gradual increase in body weight during treatment which was found to be 228.5 ± 6.96 and 197.83 ± 7.72 respectively which were comparable to the standard used. The body weight of the standard group of animals was 212.5 ± 8.77 which was reduced to 182.16± 9.99. There was gradually increase in the body weight after treatment to193.83 ± 7.38

Effect of MEMC on C-reactive protein: The CRP of different group of animals during the treatment period has been represented in Table 3. The CRP of the arthritis control group was found to be 427.40 ± 2.21. MEMC at a dose of 250 mg/kg and 500 mg/kg caused a dose dependant reduction in CRP level which was found to be 339.56 $\pm$ 2.02 and 247.86 $\pm$ 1.93 respectively which were comparable to the standard used. The standard group showed a CRP level of 178.62 ± 1.72

Effect of MEMC on copper level: The copper level of different group of animals during the treatment period has been represented in Table 4. The copper level of the arthritis control group was found to be 113.20 $\pm$ 0.20. MEMC at a dose of 250 mg/kg and 500 mg/kg caused a dose dependant reduction in copper level which was found to be 110.98 $\pm$ 0.36 and 103.16 $\pm$ 0.26 respectively which were comparable to the standard used. The copper level of standard group was 96.85 $\pm$ 0.311.

Histopathological studies: Normal animals showed bone trabeculae covered with intact articular hyaline cartilage [Fig.1) and adjacent synovial tissue. The synovial tissue consisted of synovial membrane lined by inner layer of synovial cells and outer layer of fibrofatty tissue with vascular

spaces. Arthritic control animals showed bone trabeculae covered with disrupted articular hyaline cartilage and eosinophil infltration [Fig.2]. MEMC (500 mg/kg) treated animals showed bone trabeculae covered with intact articular hyaline cartilage and adjacent synovial tissue consisted of intact synovial membrane with inner layer showing intact synovial cells [Fig.3] and outer layer showing increased fibrous tissue with vascular spaces and scattered inflammatory cells. MEMC (250 mg/kg) treated animals showed bone trabeculae covered with intact articular hyaline cartilage and adjacent synovial tissue consisted of intact synovial membrane with inner layer showing intact stratified synovial cells [Fig.4] and outer layer shows fibrofatty tissue with few vascular spaces. Animals treated with standard glucosamine showed significant inhibition of synovitis and infiltration of inflammatory cells [Fig.5].

Haematological estimation

Effect of MEMC on Haemoglobin level: The haemoglobin level of different group of animals during the treatment period is given in Table 5. The haemoglobin level of the arthritis control group was found to be reduced to 6.43 $\pm$ 0.4 when compared to normal group which showed haemoglobin level of 13.18 ± 0.06. MEMC at a dose of 250 mg/kg and 500 mg/kg caused a dose dependant increase in haemoglobin level which was found to be 13.7 $\pm$ 0.15 and 13.41 $\pm$ 0.09 respectively which were comparable to the standard used. The haemoglobin level of standard group was found to be 13.01 $\pm$ 0.37 which was similar to normal group

Effect of MEMC on RBC and WBC levels: The RBC and WBC levels of different group of animals before and after the treatment period have been represented in Table 6 and 7.

DISCUSSION

CFA induced arthritis model is one of the extensively used model to study the pathogenesis of rheumatoid arthritis for testing therapeutics. Paw swelling is one of the major factors in assessing the degree of inflammation and curative efficacy of the drug.[10] Noni treated rats showed paw edema inhibition when compared to untreated arthritis induced rats. A change in the body weight is a useful index to assess the course of the disease and the response to the therapy of anti-arthritic drugs under investigation. There was significant decrease in the body weight of arthritis rats when compared to normal rats. Administration of MEMC at the both doses used improved the body weight significantly when compared to untreated arthritis induced group. C-reactive protein is a member of the class of acute phase reactants, its levels rises dramatically during inflammatory processes. The concentration of C-reactive protein was found to be significantly reduced in the MEMC treated as well as the Glucosamine treated groups when compared to untreated arthritis induced rats. MEMC treated group of rats at 500 mg/kg showed more significant activity when compared to dose of 250 mg/kg treated group. Ceruloplasmin, an enzyme synthesized in the liver, contains 8 atoms of copper in its structure. Free copper ions are powerful catalysts of free radical damage. By binding copper, ceruloplasmin prevents free copper ions from catalyzing oxidative damage. Increased copper ion concentrations indicate the extent of an inflammatory condition. The concentration of serum copper was measured in all the groups. The arthritic rats exhibited significantly elevated copper levels, which were suppressed in noni extract and Glucosamine Sulphate treated group. However animals treated with 500 mg/kg, showed more significant reduction of serum copper when compared to the 250 mg/kg treated group. Histopathological studies support the anti-arthritic activity of the extracts. The Preliminary phytochemical analysis of MEMC showed the presence of Flavonoids, glycosides and carbohydrates. The compounds such as Flavonoids are well known for its anti-arthritic and anti-inflammatory properties. Hence the activity exhibited may be attributed to the flavonoids present in the extract. Further studies are required to establish the mechanism of action.

CONCLUSION

The result of the present work has shown significant anti-arthritic activity of *Morinda citrifolia* fruit extract in experimental CFA induced arthritis rat model. Hence, the research justified that noni extract exhibited anti-arthritic potential and hence can be further studied to isolate and characterize the active components responsible for the activity and finding can be confirmed by performing clinical studies.

Table 1: Effect of MEMC on Paw volume in CFA induced arthritic rats

Treatment Groups	Evaluation of Paw volume, mL				
	1st Day	7th Day	14th Day	21st day	28th Day
Normal	0.76± 0.021	0.76 ± 0.02	0.76 ± 0.021	0.76 ± 0.051	0.76 ± 0.021
Arthritic control	0.76 ± 0.021	1.11 ± 0.06	1.31 ± 0.047	1.36 ± 0.098	1.5 ± 0.068
MEMC-250 mg	0.73 ± 0.03^{ns}	1.23± 0.061***	1.56± 0.049***	1.46± 0.042***	1.36± 0.033***
MEMC -500 mg	0.73 ± 0.02^{ns}	1.26± 0.055***	1.55± 0.042***	1.43± 0.033***	1.33± 0.033***
Standard	0.76 ± 0.02^{ns}	1.20± 0.073***	1.46± 0.066***	1.36± 0.055***	1.25± 0.061***

Values are expressed as Mean ± SEM (n=6), by one way ANOVA followed by Bonferroni's multiple comparison test. ns is considered as non significant and ****p*<0.001 is considered as highly significant compared to normal group.

Table .2: Effect of MEMC on Body weight in CFA induced arthritis rats

SL.No.	Treatment Groups	Evaluation of Body Weight, g				
		1st Day	7th Day	14th Day	21st day	28th Day
1	**Normal**	181.83± 4.39	184.16± 4.37	185.16± 4.61	186.16± 4.67	188.6 ± 5.38
2	**Arthritic control**	207.66± 7.54	204.66± 7.55	203.33± 7.5	200.16± 7.67	194 ± 8.04
3	**MEMC-250 mg**	233.33± 7.53***	228 ±8.44^{ns}	211.3± 8.71^{ns}	220.66± 7.53*	228.5 ± 6.96*
4	**MEMC - 500 mg**	204.66± 8.94^{ns}	185.16± 12.79^{ns}	194.33± 7.28^{ns}	197.33± 7.17^{ns}	197.83 ± 7.72^{ns}
5	**Standard**	212.5± 8.77^{ns}	208.33± 8.14^{ns}	182.16± 9.99^{ns}	186.6± 10.56^{ns}	193.83± 7.38^{ns}

Values are expressed as Mean ± SEM (n=6), by one way ANOVA followed by Bonferroni's multiple comparison test. ns is considered as non significant and **p*<0.05 is considered as significant compared to normal group

Table.3: Effect of MEMC on C-reactive protein level in CFA induced arthritic rats

Sl.No.	Treatment Groups	Evaluation of CRP levels, µg/ml
		Mean ± SEM
1	**Normal**	326.69 ± 2.26
2	**Arthritic control**	427.40±2.21
3	**MEMC-250 mg**	339.56 ± 2.02***
4	**MEMC -500 mg**	247.86 ± 1.93***
5	**Standard**	178.62± 1.72***

Values are expressed as Mean ± SEM (n=6), by one way ANOVA followed by Bonferroni's multiple comparison test. ****p*<0.001 is considered as highly significant compared to arthritic control group.

Table.4: Effect of MEMC on copper level in CFA induced arthritic rats

Sl.No.	Treatment Groups	Evaluation of Cu levels, µg/ml
		Mean ± SEM
1	Normal	108.46 ± 4.6
2	Arthritic control	113.20 ± 0.20
3	MEMC-250 mg	110.98 ± 0.36***
4	MEMC -500 mg	103.16 ± 0.26***
5	Standard	96.85 ± 0.311***

Values are expressed as Mean ± SEM (n=6), by one way ANOVA followed by Bonferroni's multiple comparison test. ****p*<0.001 is considered as highly significant compared to arthritic control group.

Table 5: Effect of MEMC on Haemoglobin (Hb) level in CFA induced arthritic rats

Sl.No.	Treatment Groups	Evaluation of Hb levels, µg/ml
		Mean ± SEM
1	Normal	13.18 ± 0.06
2	Arthritic control	6.43 ± 0.4
3	MEMC-250 mg	13.7 ± 0.15***
4	MEMC -500 mg	13.41 ± 0.09***
5	Standard	13.01 ± 0.37***

Values are expressed as Mean ± SEM (n=6), by one way ANOVA followed by Bonferroni's multiple comparison test. ****p*<0.001 is considered as highly significant compared to normal group.

Table.6: Effect of MEMC on RBC level in CFA induced arthritic rats

Sl.No.	Treatment Groups	Evaluation of RBC levels, $x10^6/mm^3$
		Mean ± SEM
1	Normal	6.97 ± 0.11
2	Arthritic control	5.17 ± 0.33
3	MEMC-250 mg	7.09 ± 0.33*
4	MEMC -500 mg	6.89 ± 0.16*
5	Standard	6.06 ± 0.15**

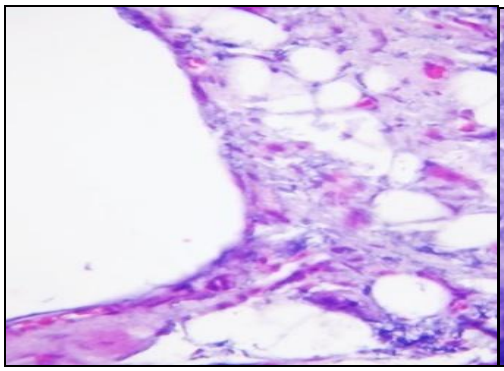
Values are expressed as Mean ± SEM (n=6), by one way ANOVA followed by Bonferroni's multiple comparison test. **p*<0.05 is considered as significant, ***p*<0.001 is considered as highly significant compared to normal group

Table.7: Effect of MEMC on WBC level in CFA induced arthritic rats

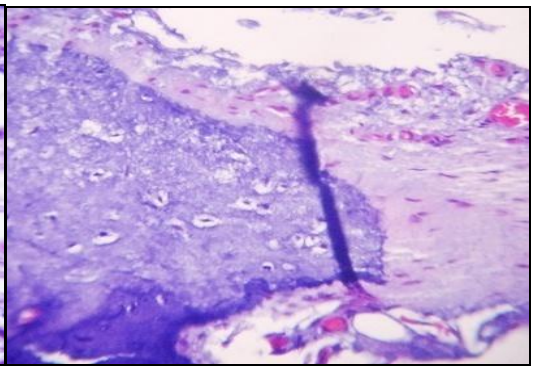
Sl.No.	Treatment Groups	Evaluation of WBC levels, $x10^6/mm^3$
		Mean ± SEM
1	Normal	6.59 ± 0.08
2	Arthritic control	11.98 ± 0.21
3	MEMC-250 mg	7.91 ± 0.22***
4	MEMC -500 mg	9.81 ± 0.04***
5	Standard	6.56 ± 0.29***

Values are expressed as Mean ± SEM (n=6), by one way ANOVA followed by Bonferroni's multiple comparison test. ****p*<0.001 is considered as highly significant compared to normal group.

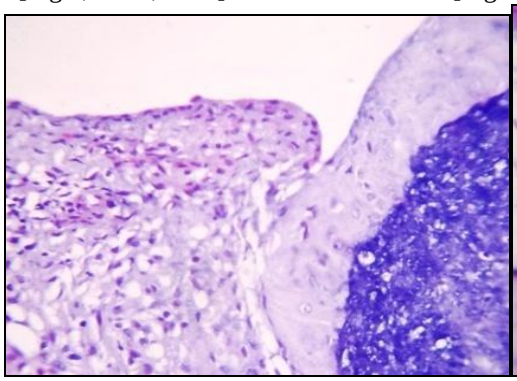
Histopathological Studies of the Antiarthritic activity of Noni extract



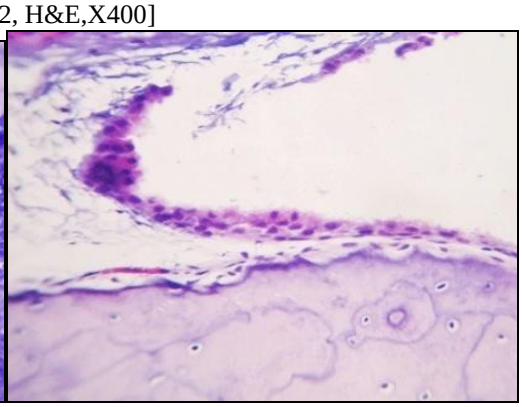
[Fig.1, H&E,X400]



[Fig.2, H&E,X400]



[Fig.3, H&E, X400]



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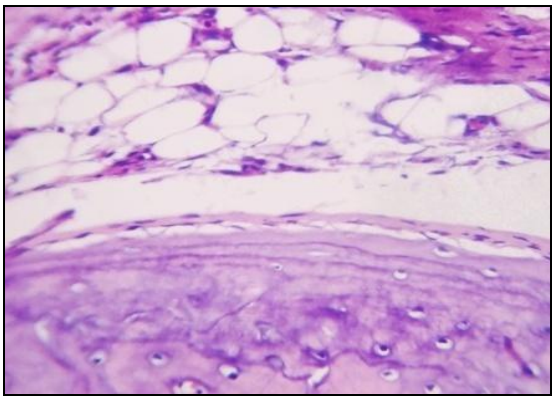

[Fig.5, H&E, X400]

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