



Anti-arthritic activity of *Morinda citrifolia* L. fruit juice in Complete Freund's adjuvant induced arthritic rats.

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Received on:10-11-2011; Revised on: 15-12-2011; Accepted on:12-01-2012

ABSTRACT

The aim of the present study was to investigate the anti-arthritic activity of *Morinda citrifolia* L. fruit juice using Complete Freund's Adjuvant (CFA) induced arthritis in rats. Arthritis was induced in rats by injecting 0.1ml CFA into the sub plantar region of the right hind limb (intradermal). Anti-arthritic activity was evaluated by measuring difference in paw thickness, arthritic index, secondary lesions, body weight and biochemical changes, supported by changes in histopathology of hind limb. Arthritic rats were orally administered with *Morinda citrifolia* fruit juice at doses 1.8ml/kg and 3.6 ml/kg between day 14-20 showed a dose dependent significant reduction in paw thickness, arthritic index, secondary lesions, increase in body weight and decreased levels of Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine transaminase (ALT), reduction in mononuclear infiltration and pannus formation when compared to CFA induced arthritic rats. Similar changes were seen with Indomethacin administration but produced no changes on secondary lesions. Anti-arthritic activity of *Morinda citrifolia* fruit juice may be due to the presence of various phyto-constituents like flavonoids, phenols, etc...

Key words: *Morinda citrifolia*, anti-arthritic, Complete Freund's Adjuvant, pannus.

INTRODUCTION:

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic, systemic inflammation affecting many tissues and organs mainly attacking synovial joints which produces an inflammatory reaction at the synovium (synovitis) and development of pannus in the synovium. Pathology of the disease process often leads to the destruction of articular cartilage and bone erosion.^[1-2] RA is one of the most prevalent health care problem and a common cause for disability. Around 1% of world population suffer from RA and female : male is 3:1.^[3-4] RA is a frequent inflammatory condition being classically treated with anti-inflammatory and immune suppressive drugs, whose side effects are well known.^[5] It would therefore be highly desirable to find less toxic alternatives. Some medicinal botanicals might be candidates for such an alternative.

Morinda citrifolia (Rubiaceae) commonly known as Noni or Indian mulberry has been used as a folk medicine and is reported to have broad range of therapeutic activity.^[6] *Morinda citrifolia* fruit juice is used for different kinds of illness such as arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, AIDS, cancer, gastric ulcer, atherosclerosis, etc. A scientific evidence of Noni fruit juice in arthritis is limited hence the present study was undertaken to investigate the potential anti-arthritic effect of *Morinda citrifolia* fruit juice. Complete Freund's Adjuvant (CFA) induced arthritis in rats bears a close resemblance to RA in humans.^[7]

MATERIAL & METHODS:

Morinda citrifolia fruit juice:

Morinda citrifolia (Noni) fruit juice was made available from a market product Herbal Noni premium no. 1(batch no. NP101), manufactured by G.N. Labs, Tilpat, Faridabad. Promoted by Herbal Ayurveda & Research center.

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Drugs and chemicals:

All the drugs used in this study were of pharmaceutical grade. Complete Freund's Adjuvant was supplied by Sigma Aldrich. Indomethacin was a gift sample from Microlabs, Bangalore, India.

Animals:

Experimental study was carried out using adult female Wistar Albino rats weighing between 160-170g. The animals were procured from Drug Testing Laboratory, Bangalore. The animals were housed in polypropylene cages. The cages were maintained clean and hygiene. Animals were acclimatized in light and temperature controlled room with a 12-12h dark-light cycle, temperature 22±2°C and humidity 50±5%. The rats were fed with commercial pelleted rat feed and water *ad libitum*. The Institutional Animal Ethics Committee (IAEC/NCP/11/09) at Nargund college of pharmacy had approved the study.

Experimental induction of arthritis:^[7,8,9]

Complete Freund's Adjuvant induced arthritic model was used to assess the anti-arthritic activity of *Morinda citrifolia* fruit juice in Wistar rats. Arthritis was induced in the rats by injecting 0.1ml CFA containing (5mg/ml of heat killed *Mycobacterium tuberculosis*) into the sub plantar region of the right hind limb (intradermal) on day 0. Two doses of *Morinda citrifolia* fruit juice 1.8ml/kg b.w. and 3.6ml/kg b.w. were selected based on human dosage. Indomethacin (3mg/kg b.w.) in 2% w/v gum acacia was selected.^[10]

Rats were divided into five groups of 6 animals each, as follows:

Group-I: Vehicle control (saline 5ml/kg b.w.)

Group-II: Positive control (CFA 0.1ml)

Group-III: CFA induced arthritic rats were administered orally with Indomethacin from day 14 to 20.

Group-IV: CFA induced arthritic rats were administered orally with *Morinda citrifolia* fruit juice (1.8ml/kg) from day 14 to 20.

Group-V: CFA induced arthritic rats were administered orally with *Morinda citrifolia* fruit juice (3.6ml/kg) from day 14 to 20.

Assessment of arthritis:

Arthritis was assessed by physical and biochemical parameters further sup-

ported by histopathology of the hind limb.

a) Physical assessment:

Initial and successive body weights and paw thickness were measured and recorded up to day20. Paw thickness was assessed by the measurement of right hindpaw (ipsilateral) using vernier scale. Secondary lesions on the non-injected hindpaw, forepaws, tail and nose were noted. Arthritic index was calculated on day20.⁽¹¹⁾

$$AI (%) = \frac{\text{Hind paw vol. day20} - \text{Hind paw vol. day0}}{\text{Hind paw vol. day0}} \times 100$$

b) Biochemical assessment:

Animals were sacrificed by cervical dislocation on day 20; blood was collected from various groups. Serum was separated and estimated for ALP, AST and ALT levels.

c) Histopathological assessment:

The injected paws were isolated and preserved in 10% v/v buffered formalin solution. The tarsal-metatarsal joints were excised for paraffin embedding. Sections were taken on the slides by microtomy and were stained with Hematoxyline-Eosin (H&E) stain.

Statistical analysis:

The results were expressed as mean±SEM and analyzed statistically by ANOVA followed by Dunnett's t test.

RESULTS

A) Physical examination:

a) Paw thickness and Arthritic Index:

Fig.1 and Fig.2 showed significant (P<0.001) increase in paw thickness and arthritic index in CFA induced arthritic rats when compared to vehicle control group. Arthritic rats treated with Indomethacin and *Morinda citrifolia* fruit juice showed significant (P<0.001) decrease in paw thickness and arthritic index when compared to CFA induced arthritic group.

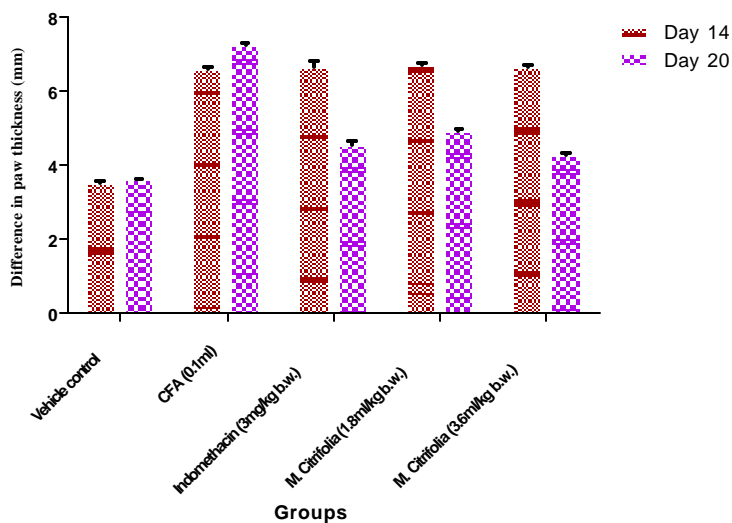


Fig. 1: Changes in paw thickness of rats after treatment from day14-20. Data were analysed by one way ANOVA followed by Dunnett's t test. Number of animals in each group n = 6.

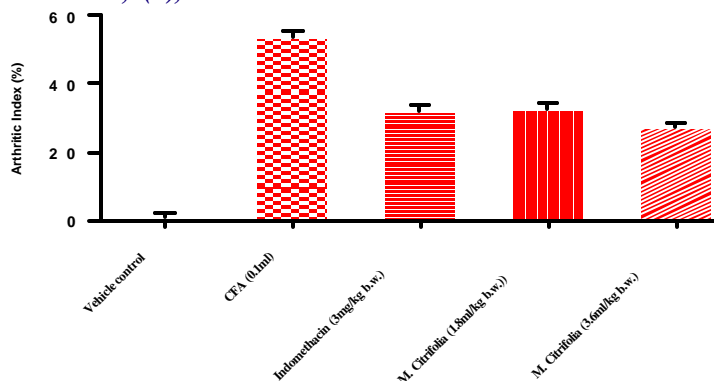


Fig. 2: Changes in Arthritic Index of rats after treatment from day14-20. Data were analysed by one way ANOVA followed by Dunnett's t test. Number of animals in each group n = 6.

b) Body weight examination:

CFA induced arthritic rats showed a significant (P<0.001) decrease in body weight when compared to vehicle control group. Arthritic rats treated with Indomethacin and *Morinda citrifolia* fruit juice showed significant (P<0.001) increase in body weight when compared to CFA induced arthritic rats (Table No. 1).

Table no.1: Effect of *Morinda citrifolia* fruit juice on body weight (g) in CFA induced arthritic rats.

Groups	Vehicle control (Mean±SEM)	Complete Freund's Adjuvant (CFA) (0.1ml) (Mean±SEM)	CFA (0.1ml) + Indomethacin (3 mg/kg b.w.) (Mean±SEM)	CFA (0.1ml) + <i>Morinda citrifolia</i> (1.8ml/kg b.w.) (Mean±SEM)	CFA (0.1ml) + <i>Morinda citrifolia</i> (3.6ml/kg b.w.) (Mean±SEM)
Body weight on Day0	161.2±0.24	160.7±0.16	160.8±0.30	161.3±0.38	160.9±0.23
Body weight on Day14	185.0±0.64	164.0±0.31	163.3±0.44	163.5±0.40	163.8±0.42
Body weight Day20	195.8±0.69	163.3±0.33***a	183.2±0.38****b	178.9±0.65****b	187.9±0.67****b
Increase b.w. (Day14-20)	11.17±0.84	0.34±0.51****a	19.75±0.38****b	15.08±0.52****b	24.42±0.52****b

Data were analysed by one way ANOVA followed by Dunnett's t test. Number of animals in each group n = 6. ^a comparison made with vehicle control group. ^b comparison made with CFA induced group. **** P<0.001.

a) Secondary lesions:

CFA induced arthritic rats showed the presence of secondary lesions on non injected left hind paw, fore paws, tail and nose. Arthritic rats treated with *Morinda citrifolia* fruit juice showed no secondary lesions, whereas arthritic rats after treatment with Indomethacin showed secondary lesions (Table No. 2)

Table no.2: Effect of *Morinda citrifolia* fruit juice on secondary lesions in CFA induced arthritic rats.

Groups	Non injected left hind paw	Fore paws	Tail	Nose
Vehicle control	Absent	Absent	Absent	Absent
CFA induced arthritis	Present	Present	Present	Present
CFA + Indomethacin	Present	Present	Present	Present
CFA + <i>Morinda citrifolia</i> (1.8ml/kg)	Absent	Absent	Absent	Absent
CFA + <i>Morinda citrifolia</i> (3.6ml/kg)	Absent	Absent	Absent	Absent

(B) Serum biochemical estimations:

CFA induced arthritic rats showed a significant increase ($P < 0.001$) in ALP, AST and ALT levels when compared to vehicle control group. Arthritic rats treated with Indomethacin and *Morinda citrifolia* fruit juice showed significant ($P < 0.001$) decrease in ALP, AST and ALT levels when compared to CFA induced arthritic rats (Table No. 3)

Table no.3: Effect of *Morinda citrifolia* fruit juice on Alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine transaminase (ALT) in CFA induced arthritic rats.

Groups	ALP	AST	ALT
Vehicle control	257.5±9.57	131.3±3.66	39.59±1.05
CFA induced arthritis	803.1±39.70**** ^a	185.8±5.00**** ^a	78.14±0.82**** ^a
CFA + Indomethacin	440.4±28.50**** ^b	163±0.5**** ^b	56.42±0.67**** ^b
CFA + <i>Morinda citrifolia</i> (1.8ml/kg)	566.7±38.14**** ^b	168±0.88**** ^b	62.0±0.7**** ^b
CFA + <i>Morinda citrifolia</i> (3.6ml/kg)	376.9±6.46**** ^b	154.4±1.09**** ^b	45.09±1.5**** ^b

Data were analysed by one way ANOVA followed by Dunnett's t test. Number of animals in each group n = 6. ^a comparison made with vehicle control group. ^b comparison made with CFA induced group. **** $P < 0.001$.

c) Histopathological studies:

Section studied from the Tarsal-Metatarsal joint in control rats showed intact articular hyaline cartilage and synovial lining cells within normal range (Fig.3). Arthritic rats showed proliferation of synovial lining cells, diffuse infiltration of lymphocytes and plasma cells and also pannus formation in focal areas (Fig.4). Administration of Indomethacin (Fig.5) and *Morinda citrifolia* fruit juice (1.8ml/kg) to CFA induced arthritic rats showed presence

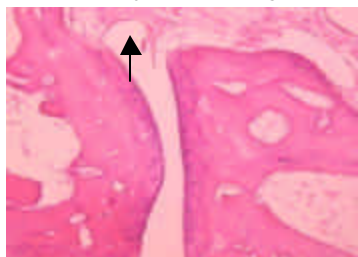


Fig. 3: Section of Tarsal-Metatarsal joint in control rats (H&E, 100x) showing normal tissue architecture.

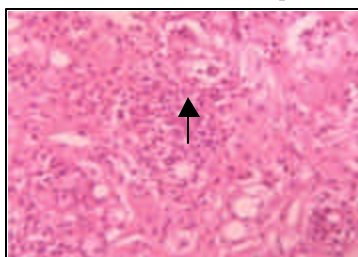


Fig. 4: Section of Tarsal-Metatarsal joint of CFA induced arthritic rats. (H&E, 400x) showing aggregates of lymphocytes and macrophages.



Fig. 5: Section of Tarsal-Metatarsal joint of CFA induced arthritic rats. (H&E, 400x) showing few villus membrane like projections and fibrocollagenous stroma.

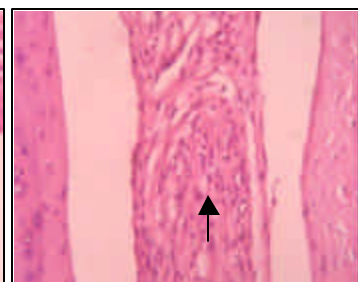


Fig. 6: Section of Tarsal-Metatarsal joint of CFA induced arthritic rats. (H&E, 400x) showing infiltration of lymphocytes and plasma cells.

of few villus like projection of synovium, scattered subintimal infiltration by plasma cells and lymphocytes (Fig.6). Administration of *Morinda citrifolia* fruit juice (3.6ml/kg) to CFA induced arthritic rats showed focal aggregates of plasma cells and lymphocytes, plump endothelial lining of blood vessels (Fig.7).

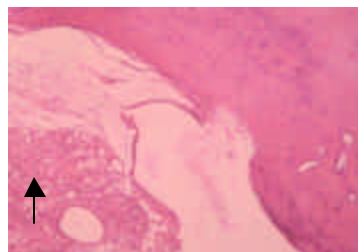


Fig. 7: Section of Tarsal-Metatarsal joint in control rats (H&E, 100x) showing normal tissue architecture.

DISCUSSION

Complete Freund's Adjuvant induced arthritis in rats is an experimental model that mimic many of the clinical and pathological features of human Rheumatoid Arthritis such as swelling, bone erosion, cartilage degradation and loss of joint functions.^[12]

Increased paw thickness in CFA induced arthritic rats was due to oedematic swelling and increased infiltration of granulocytes and monocytes at the inflamed joint.^[7] Paw oedema is one of the major parameters for assessing the degree of inflammation.^[13]

Intradermal injection of CFA into right foot pad of the rat produces inflamed primary lesions at the site of injection. After a delay of approximately 13 days inflamed secondary lesions appear in remote areas of the body such as non-injected left hindpaw, fore paws, tail and nose.^[8]

Change in body weight is also a parameter to assess the severity of arthritis. Decrease in body weight is observed in arthritic rats due to alteration in metabolic activities of rats and also due to reduced absorption of glucose and leucine in rat intestine.^[14] In addition to this decreased food intake throughout the study period due to partial immobility accompanying hyperalgesia may also be one of the reason. Administration of Indomethacin and *Morinda citrifolia* fruit juice to CFA induced arthritic rats showed a significant increase in body weight which may be due to overcoming the altered metabolic process following CFA induction.

Secondary lesions on the non-injected hindpaw, forepaws, tail and nose were seen in arthritic group as a result of generalized immunological response to constituents of tubercle bacilli, which have become disseminated after injection.^[8] Secondary lesions were not seen in groups treated with *Morinda citrifolia* fruit juice indicating the suppression of immunomodulatory mediated inflammatory process. Though there is a significant decrease in paw volume, secondary lesions were not significantly reduced after administration of Indomethacin to arthritic rats which indicates Indomethacin inhibits the primary inflammatory process but not mediated by immune reaction.

Alterations in serum levels of ALP, AST and ALT are used as sensitive markers of cellular integrity and cellular toxicity induced by pathological conditions.^[4,7] Alterations in these levels are more specific to inflammatory conditions. It also acts as an indicator for phagocytic activity. Increase in serum ALP, AST and ALT levels were seen in CFA induced arthritic rats due to release of enzymes from the damaged liver cells - a feature of CFA induced arthritis.^[7] Decreased levels were seen with Indomethacin and *Morinda citrifolia* fruit juice administered to CFA induced arthritic rats showing a significant liver protection.

Histopathological examination revealed normal architecture of tarsal-metatarsal joints in vehicle control group. In CFA induced arthritic rats, oedematic swelling in the form of synovial hyperplasia, pannus formation, diffuse subintimal infiltration by plasma cells and lymphocytes, plump endothelial lining of blood vessels were seen.^[4,7] Arthritic animals treated with Indomethacin and *Morinda citrifolia* fruit juice showed almost towards the normal architecture of the joints.

From the above experimental studies carried out, *Morinda citrifolia* fruit juice at two different orally administered doses (1.8ml/kg b.w. and 3.6ml/kg b.w.) showed dose dependent anti-arthritis activity in the treatment of CFA induced arthritis. The anti-arthritis effect may be due to the presence of various phyto-chemical constituents like Flavonoids, Phenolic compounds etc. present in *Morinda citrifolia* fruit juice. Further studies need to be carried

out to isolate the potential phyto-chemical constituents present in *Morinda citrifolia* fruit juice and know its mechanism of action in the treatment of rheumatoid arthritis.

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Source of support: Nil, Conflict of interest: None Declared