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(RESEARCH ARTICLE)

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Assessment of anti-arthritic efficacy of the ethanolic extract of the entire *Morinda citrifolia* Plant

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Abstract

The study investigates the anti-arthritic and anti-inflammatory properties of an ethanol extract of the entire *Morinda citrifolia* plant in a rat model of arthritis caused by Complete Freud's Adjuvant (CFA). The extract's effectiveness was assessed by assessing key indicators of inflammation and arthritis. The results showed a substantial reduction in inflammation and arthritis-related symptoms in the treatment group, indicating that the extract had potent anti-inflammatory capabilities. The findings point out the therapeutic potential of *Morinda citrifolia*, a natural arthritis treatment, which may be attributable to active phytochemicals such as flavonoids and alkaloids. More research is needed to explain the underlying processes and investigate their potential application in treating human arthritis.

Keywords: *Morinda citrifolia*; Anti-arthritic activity; Anti-inflammatory effects; Complete Freund's Adjuvant (CFA); Ethanol extract; Phytochemicals; Flavonoids and alkaloids; Rheumatoid arthritis model

1. Introduction

Rheumatoid arthritis is a chronic, systemic inflammatory disorder or a long-term auto-immune multisystem illness in which the body's immune system attacks the body's tissues and joints mistakenly causing inflammatory synovitis which often progresses the destruction of joint ankylosis and articular cartilage [1]. An autoimmune disease is a condition that arises from an abnormal response to our normal immune system. The immune system is a host defence mechanism comprising a complex organization of cells and antibodies designed normally to "seek and destroy" invaders of the body. The synovium (inside of joints) is a thin delicate lining that serves as an important source of nutrients for cartilage which thickens during RA resulting in inflammation and pain in and around the joints. Additionally, synovial cells synthesize joint lubricants and help them move smoothly such as collagens, as well as fibronectin and hyaluronic acid that constitute the structural framework of the synovial interstitial [2].

The current pharmacologic therapies for the treatment of RA are NSAIDs, Glucocorticoids, DMARDs, and other antirheumatic drugs.

Morinda citrifolia L. (Rubiaceae), commonly known as Noni, has been extensively used in folk medicine by the Polynesians for over 2000 years. It is having high demand as an alternative medicine due to its use as an antioxidant, anti-fungal, anti-bacterial, anti-inflammatory, liver protective, anticancer, analgesic, immunomodulatory, anti-viral and wound healing effects etc. Phytochemical investigations have shown that noni contains many phenolic compounds, in particular, coumarins, flavonoids, and iridoids which are reported to have various pharmacological effects.

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2. Methods

2.1. Preparation of extracts

About 750 grams of powdered whole plant of *Morinda citrifolia* (Linn.) was taken in a 5000 ml round bottom flask and extracted using a cold extraction method for 12 days using ethanol (50%) with occasional stirring. The ethanol extract was filtered through Whatman filterpaper to remove the impurities present. The ethanolic extract was concentrated by vacuum distillation, cooled and placed in a desiccator to remove the excessive moisture. Quantity of extract obtained-154.21 grams from 750 grams of the raw material.

2.2. *In-vivo* anti-arthritic study

2.2.1. Induction of Arthritis

To induce arthritis, animals were first anaesthetized with a small amount of ether vapour, then a single injection of 0.2 ml Complete Freund's adjuvant dissolved in mineral oil (sterile) was injected delicately into the sub-plantar region of the left hind paw

2.2.2. Treatment Regimen

- Group I - Animals are given normal saline/ p.o.
- Group II - Complete Freund's adjuvant-induced Arthritis animals treated with standard, 0.75 mg/kg Methotrexate/ p.o.
- Group III – Complete Freund's adjuvant-induced Arthritic animals treated with 200 mg/kg of *Morinda citrifolia* (Linn.), p.o;
- Group IV – Complete Freund's adjuvant-induced Arthritic animals treated with 400 mg/kg of *Morinda citrifolia* (Linn.), p.o; Vehicle control animals were given 1% w/v of DMSO solution daily.

The drug treatment was continued with the respective groups for 40 days.

Every day animals were carefully and thoroughly inspected by examining the affected paw and animal's general status. The health status parameters included paw volume, animal body weight, arthritic score and behavioural observations such as locomotor activity. The body weights of all the animals were recorded in grams every week by using a single pan weighing balance. Body movement was measured by observing the time taken by individual animals to move a 2-meter distance and statistical analysis was performed. The animals were sacrificed on day 41 to study the histology of the joint.

2.2.3. Paw edema volume

The hind paw volumes of all the animals were measured just before Freund's complete adjuvant injection on day 0 and after that at different time intervals (day 4, 13, 25, 40) using a plethysmometer instrument [12, 13 & 14].

2.3. In-vitro anti-arthritic study

2.3.1. Protein denaturation

Procedure

100µl of the test was added with 500µl of 1% BSA. The mixture was incubated for 10 minutes at 37ºC. Heat the contents in a water bath at 51ºC for 20 minutes. Cool down to room temperature and check the absorbance at 660nm against the blank. Acetyl Salicylic acid was used as positive control and water as product control. Mizushima Y *et al.*

$$\% = 100-\{(A1-A2)/A0*100\}$$

2.4. Statistical analysis

The data was analyzed in terms of Mean ± Standard error of Mean (SEM). For statistical analysis,multiple comparisons of data were made using one and two way analysis of variance (ANOVA) followed by Dunnet‟s test was used for post hoc analysis. Significance wasstatistically acceptableat a level of $P < 0.05$. Software program GraphPad Prism was used for all data analysis.

3. Results

3.1. *In vivo* results

3.1.1. Paw edema volume

In FCA induced arthritis model, rats developed chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage, bone destruction and remodeling. These inflammatory changes ultimately result in the complete destruction of joint integrity and functions in the affected animal.

The extract of *Morinda citrifolia Linn.* at 400 mg/kg inhibited rat paw edema which is comparable with the standard drug methotrexate on the 40th day. The results of which are shown in Table 1. The determination of rat paw swelling is apparently simple, sensitive and one of the quick procedures for evaluating the degree of inflammation and the therapeutic effects of drugs. Chronic inflammation involves the release of a number of mediators like cytokines, GM-CSF, interferons and PGDF. These mediators are responsible for pain and destruction of bone, and cartilagethat can leads to severe disability.

3.2. Invitro results

3.2.1. Protein Denaturation

Anti–arthritic effect of EMP extract was studied significantly by testing various in–vitro parameters. Table 2 depicts the inhibition of protein denaturation of different extracts. In the present investigation, all three extracts inhibited the protein denaturation in a dose-dependent manner. However, the EMP has a higher inhibitory percentage of protein denaturation when compared ($p>0.05$) to the positive control. At the concentrations of 400, 500, 800 and 1000µg/ml, the inhibitory percentage of EMP was significantly comparable to the positive control Acetyl salicylic acid used in this present investigation.

Table 1 Effect of *Morinda citrifolia* Linn Extracts On Changes In PawVolume In Cfa-Induced Arthritis In Rats

Group	**0^{th} day**	**9th day**	**18th day**	**25th day**	**40th day**
Control	0.65 ± 0.01	0.66 ± 0.01	0.65 ± 0.02	0.67 ± 0.01	0.67 ± 0.02
Negative control	0.66 ± 0.02	0.75 ± 0.01	0.80 ± 0.01	0.86 ± 0.02	0.91 ± 0.02
Standard	0.59 ± 0.01	0.48 ± 0.01	0.43 ± 0.02	0.40 ± 0.01	0.35 ± 0.02
200 mg/kg MP	0.62 ± 0.02	0.51 ± 0.01	0.42 ± 0.02	0.40 ± 0.01	0.38 ± 0.01*
400 mg/kg MP	0.58 ± 0.02	0.42 ± 0.01	0.36 ±0.01*	0.32 ±0.01**	0.27 ± 0.01*

Values are expressed in mean ± SEM, n = 6, *p<0.05 are significant compared to standard,LDMC (200 mg/kg); HDMC (400 mg/kg)

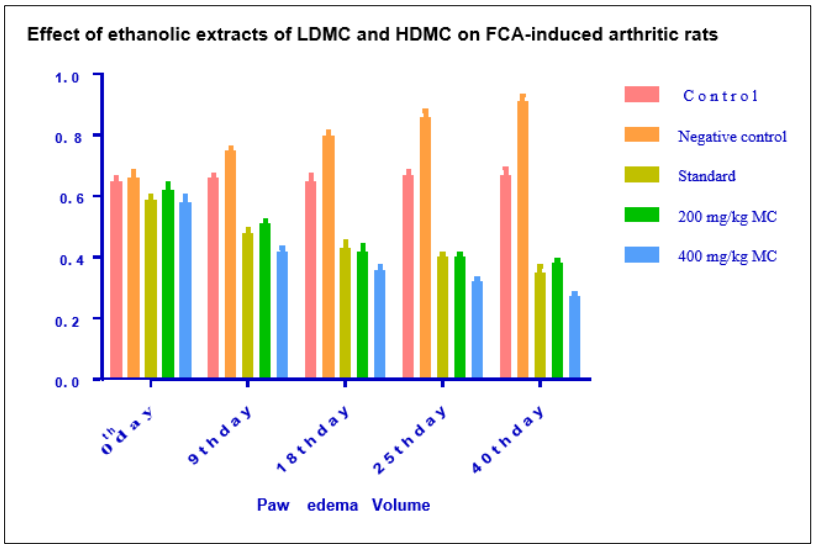

Figure 1 Effect of ethanolic Extracts of LDMC and HDMC FCA-Induced Arthritic Rats

Table 2 Protein Denaturation Study

Concentration (mg/ml)	Inhibitory activity of EMC	Inhibitory activity of Acetyl Salicylic Acid (%)
100	24.46 ± 1.12	24.45 ± 1.70
200	33.62 ± 3.08	32.14 ± 2.21
400	45.76 ± 1.98	41.77 ± 1.52
500	54.35 ± 2.37	45.33 ± 2.18
800	76.48 ± 1.92	69.71 ±2.43*
1000	87.65 ± 3.01	85.17 ±2.13*

Values are expressed in mean ± SD (n=6), *p<0.05. A statistical significant test for comparison was done by ANOVA followed by Dunnet's 't' test. Comparison between acetylsalicylic acid vs EMC

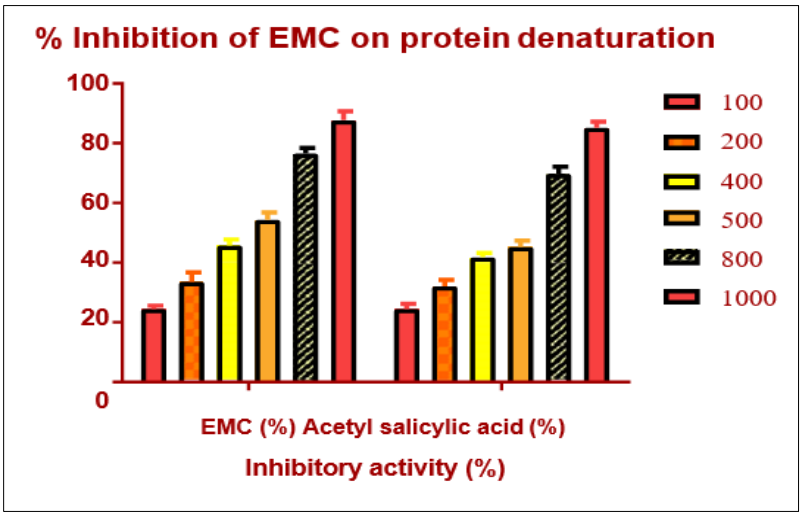

Figure 2 %Inhibition of EMC on protein denaturation

4. Discussion

Rheumatoid arthritis is an inflammatory, autoimmune disorder that destroys the immune system. The immunologically mediated Complete Freund's adjuvant-induced arthritic model of chronic inflammation is considered the best available experimental model of rheumatoid arthritis [70]. Complete Freund's adjuvant-induced arthritis is a model of chronic polyarthritis with features that resemble rheumatoid arthritis.

In Complete Freud's adjuvant-induced arthritis model, rats developed chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage and bone destruction and remodelling which have close similarities to human rheumatoid disease. These inflammatory changes ultimately result in the destruction of joint integrity and functions in the affected animal. Also, Complete Freund's adjuvant-administered rats showed soft tissue swelling around the ankle joints during the development of arthritis, which was considered as edema of the particular tissues.

Paw swelling is an index of measuring the anti-arthritic activity of *Morinda citrifolia* Linn. at the dose level 200&400 mg/kg, p.o. *Morinda citrifolia*-administered groups showed a marked reduction in paw volume when compared with the Negative control group (Group II). It was also found that there was significant weight loss when compared to standard [71]. The result of the present study also indicates that there is a close relationship between the extent of inflammation, loss of body weight and arthritic index. The arthritic scoring was done based on visual observation where it can be seen that there is a marked reduction in the swelling and joint damage of the drug-treated groups [72]. It was also noted that the high dose of *Morinda citrifolia* Linn. Extract proved its efficacy in reducing the inflammation of the paws. The locomotor activity of the animals was improved in Group 5 animals (HDMP) when compared to the standard animals.

5. Conclusion

In conclusion, this study has verified that constituents of the plant suppressed joint inflammation and destruction in adjuvant arthritic rats. We are confident that our data provide mechanistic evidence for the anti-arthritic appliance of the plant as a promising candidate for novel therapeutic agents of Rheumatoid arthritis.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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