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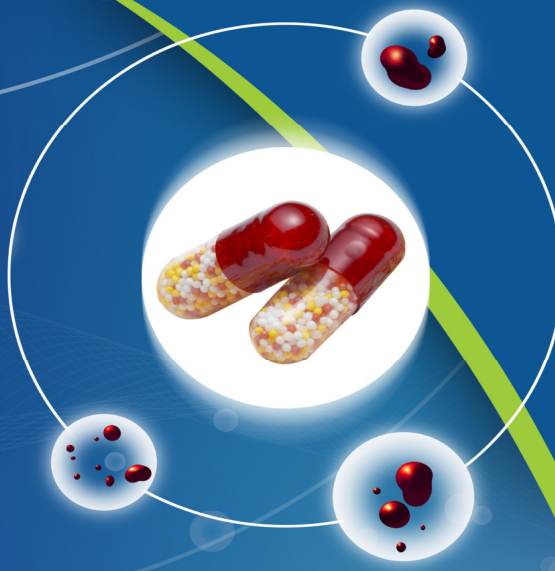

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Research Article

PROPHYLACTIC EFFECT OF *AEGLE MARMELOS* LEAVES AGAINST COMPLETE FREUNDS ADJUVANT (CFA) INDUCED ARTHRITIS

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic crippling, skeleton-muscular disorder having nearest approximation to human rheumatoid arthritis for which there is currently no medicine available effecting a permanent cure. Even modern drugs used for the amelioration of the symptoms, offer only temporary relief and also produce severe side effects. This work was aimed at the scientific validation of the ethno-pharmacological claim about Aegle marmelos and its anti-arthritic property. In the present study, anti-arthritic activity of aqueous extract of Aegle marmelos (AEAM) is done by prophylactic model of Complete freunds adjuvant (CFA) induced arthritis. Parameters such as paw edema, paw diameter and changes in body weight during arthritic condition was corrected on treatment with AEAM. Serum parameters such as SGOT, SGPT, ALP, and Total protein were also estimated for assessing the anti- arthritic potential of AEAM. The results of the current investigation concluded AEAM possess a significant anti-arthritic activity against CFA induced arthritis model and justifying its therapeutic role in arthritic condition. The observed antiarthritic activity may be due to the presence of phytoconstituents such as alkaloid and flavonoids.

Keywords: *Rheumatoid Arthritis, Aegle marmelos, Complete freunds adjuvant.*

INTRODUCTION

Rheumatoid arthritis (RA) is a complex autoimmune disease characterised by persistent inflammation of the synovium, local destruction of bone and cartilage and a variety of systemic manifestations which may ultimately result in functional disability.

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Autoimmune diseases occur when the body looses the ability to discriminate self proteins from non-self proteins. This loss of tolerance ultimately results in the destruction of self tissues by the immune system. Typically, autoimmune diseases are characterized by the presence of auto-antibodies and autoreactive T-lymphocytes acting against specific self proteins [1]. Within joints, the autoimmune mediated course of RA is characterized by four stages: (I) inflammation of the synovial membrane and joint capsule, (II) formation of a pannus (granulation tissue) that first covers and then invades cartilage and bone, (III) fibrous invasion of the pannus, (IV) calcification of the fibrous

tissue [2].The goals of therapy of RA are (I) relief of pain, (II) reduction of inflammation, (III) protection of articular structures, (IV)maintenance of function, and (V)control of systemic involvement. Since the etiology of RA is unknown, the pathogenesis is not completely delineated, and the mechanisms of action of some of the therapeutic agents employed are uncertain, therapy remains somewhat empirical. None of the therapeutic interventions is curative, and therefore all must be viewed as palliative, aimed at relieving the signs and symptoms of the disease. The various therapies employed are directed at nonspecific suppression of the inflammatory or immunologic process in the hope of ameliorating symptoms and preventing progressive damage to articular structures [3]. Even though various categories like immunosuppressants, NSAIDs, steroidal anti inflammatory drugs are being used till now, but the potential side effects give a limitation for their use. Traditional medicines derived mainly from plants play major role in the management of arthritis as they are effective, non-toxic, with less or no side effects and are considered to be excellent candidates for arthritic therapy [4]. The plant products and their combinations are running well now in the market for the treatment of inflammatory and autoimmune diseases due to their lower side effects, efficacy and less cost. Now it is a growing concern allover for the development of new safe, potent, less toxic anti-arthritic drug. Hence, there is a need to explore for more naturally available alternatives, so that their therapeutic values can be assessed and expanded [5]. *Aegle marmelos* commonly known as bael, belongs to the family rutaceae, and is a cosmopolitan plant distributed throughout tropical asia and africa. The principle chemical constituents of leaves extract are tannins, skimmianin, essential oil (mainly caryophyllene, cineole, citral, citronellal, d-limonene and eugenol), sterols and triterpenoids, including lupeol, sitosterol, and amyrin, flavonoids (mainly Rutin, flavones) and coumarins including aegeline, marmesin and umbelliferone. The Bael leaves are bitter and used as a remedy for opthalmia, ulcers, inflammations, dropsy, cholera and beriberi. A decoction of plant leaves and fruit is used in upper respiratory tract infections and heart ailments. Leaves are also reported for hypoglycaemic effect, antioxidant, anticarcinogenic and anti-inflammatory properties [6].

Most of anti-inflammatory plants shows anti-arthritic activity and presence of phytochemicals such as alkaloids, flavonoids, steroids are responsible for anti-arthritic activity [7]. Recent studies conclude that aqueous extract of *Aegle marmelos* leaves shows presence of phytochemicals such as alkaloids, flavonoids, steroids [8]. Also, *Aegle marmelos* plant have traditional claim for use in inflammatory disorder [9]. But no pharmacological work has been done on evaluation of its anti-arthritic activity. So the present study was carried out to evaluate anti-arthritic effect of aqueous extract of *Aegle marmelos* leaves in male wistar rats.

MATERIALS

Chemicals and Drugs

Complete freunds adjuvant (CFA)- Sigma-Aldrich, USA [F-5881], Diclofenac Sodium injection, Methotrexate Injection I.P., SGOT, SGPT, ALP and Total Protein kits.

Instruments Used

Plethysmometer- UGO-Basile, Italy (7140), Autoanalyser- Lablife Chem master Ltd; New Delhi, India. Microcentrifuge- Remi motors RM- 12C; Mumbai,India., Verneir caliper- Malik tools, Mumbai, Oral feeding needle- BIK Industries, Mumbai.

Animals

Male Wistar rats (150-250 g) or female Swiss albino mice (20-25 gm) obtained from the Yash Farm and National Toxicological Centre, Pune were used for study.

Housing conditions

Animals were maintained at a temperature of 25±1°C and relative humidity of 45 to 55 % under 12 hr light and 12 hr dark cycle. The animals had free access to standard food pellets, procured from Pranav Agro Industries Ltd., Sangli, India and water *ad libitum*.

Institutional Animal Ethics Committee Approval

The experimental design and research plan along with animal handling and disposal procedure were approved from Institutional Animal Ethics Committee of Rajgad Dnyanpeeth's College of Pharmacy, Bhor. IAEC approval No: RDCOP/IAEC/2010-11/03.

METHODS

Collection and Authentication of Plant Material

The leaves of *Aegle marmelos* was collected from Bhor region of Maharashtra in the month of September-October 2010 and authenticated by Botanical Survey of India, Pune and herbarium voucher specimen No: BSI/WRC/Tech/2011/JAPAEM5.

Preparation of Aqueous Extract of Aegle marmelos (AEAM) leaves

Leaves of *Aegle marmelos* were shade dried and coarsely powdered by using grinder mixer. The powdered material was macerated in sufficient quantity of distilled water with small quantity of chloroform to prevent fungal growth and kept for 3 days. During maceration it was shaken twice daily. On 3rd day it was filtered and dried at 60 °C on water bath [10]. The extract was then preserved in the desicator and then used for phytochemical and pharmacological studies.

Phytochemical screening of the extract

AEAM was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids [11].

Acute oral toxicity study (AOT):

Healthy adult swiss mice (20-30 gm) were subjected to acute oral toxicity studies as per Organization for Economic Co-operation and Development (OECD) guidelines. Animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days.The changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system, somatomotor activity and behaviour pattern were noted [12].

EXPERIMENTAL DESIGN

Prophylactic effect of aqueous extract of Aegle marmelos in complete Freund's adjuvant induced arthritic rats.

Freund's adjuvant induced Arthritis model was used to assess the anti-arthritic activity in albino wistar rats. Animals were randomly divided into six groups of six animal each (n=6). Group Ist receives Distilled water 5 ml/kg, p.o., Group IInd receives CFA 0.1ml, s.c., Group IIIrd receives Diclofenac 10mg/kg, i.p., Group IVth, Vth, VIth, receives AEAM at the dose 100mg/kg, 200mg/kg, 400mg/kg, p.o, respectively. Wistar rats were made arthritic by single subplanter injection of 0.1 ml of Complete freund's adjuvant (CFA). Drug treatment was started from the initial day i.e. from the day adjuvant injection (0day) and continued till 21st day. Paw volume and Paw diameter was measured on 0th, 4th, 8th, 14th, 21st day by using plethysmometer and verneir caliper respectively [13, 14].

Statistical analysis

The values were expressed as mean ± SEM (n=6). The statistical significance was assessed using student t-test or one-way analysis of variance (ANOVA) followed by Dunnet's test and *P<0.05 and **P<0.01were considered to be statistically significant.

RESULT

Physical properties of AEAM

Colour :- Blackish Brown

Odour:- Characteristic

Taste:- Bitter

Appearance:- Sticky

% Yeild:- 10.23 %

Phytochemical screening of the extract

Phytochemical study of AEAM showed the presence of various phytoconstituents like alkaloids, carbohydrates, glycosides, saponins, tannins, and flavonoids.

Acute oral toxicity (AOT) of AEAM

According to OECD guidelines for acute oral toxicity at the dose of 2000mg/kg, animals in the group treated with AEAM did not showed any symptoms of toxicity at this dose level and no mortality was observed during the 14 days of observational period. Hence, according to the guideline, the different doses of AEAM selected present study for per oral administration were 100 mg/kg, 200 mg/kg and 400 mg/kg.

Prophylactic effect of aqueous extract of Aegle marmelos in complete Freund's adjuvant induced arthritic rats.

Sub planter injection of Freund's complete adjuvant in the rat hind paw led to development of arthritis which reached a peak edema on 14th day of injection. There was significant decrease in paw edema in Diclofenac (10mg/kg) treated group on day 8th, 14th and 21st with P<0.01. The result indicates that AEAM (200 mg/kg) treated group significantly suppressed paw edema on day 14th and day 21st with P<0.05 and P<0.01 respectively, whereas AEAM (400mg/kg) treated group suppressed paw edema significantly on day 8th, 14th and 21st with P<0.01 (Table 1). Percent inhibition of paw volume is also calculated, according to study Diclofenac (10mg/kg) showed 79.90%, AEAM (200 mg/kg) and AEAM (400mg/kg) showed 35.29% and 69.60% respectively **(Table 2).** Increased paw diameter, due to inflammation and edema was also observed. In the control group paw diameter showed changes up to 8th day and slightly decreased until day 21st.There was significant decrease in paw diameter in Diclofenac (10mg/kg) treated group on day 8th,14th and 21st with P<0.01. AEAM (200 mg/kg) and AEAM (400mg/kg) treated group suppressed paw diameter significantly on day 8th,14th and 21st with P<0.01 **(Table 3).**The biochemical parameters such as SGOT, SGPT, ALP were estimated for assessing the effect on liver function. There was a significantly increased in the level of SGPT, SGOT, ALP and decreased in level of Total protein is observed in control group when compared with normal. Diclofenac (10mg/kg) treated group significantly decreased the level of SGPT, SGOT, ALP and increased the level of Total protein. AEAM (200mg/kg) shows significant changes in the level of SGPT, ALP, and Total protein with P<0.05, whereas AEAM (400mg/kg) shows significant changes in the level of SGPT (P<0.05), SGOT (P<0.05), ALP (P<0.01) and Total protein (P<0.01) **(Table 4).**

Sub planter injection of CFA shows significant decreased in body wt when compared with normal group. Diclofenac Sodium treated group was found to be significant in body with P < 0.01.Whereas AEAM (200mg/kg) and AEAM (400mg/kg) show significant increased in body wt with P < 0.05 and P < 0.01 respectively **(Table 5).**

Table 1: Effect of AEAM on CFA induced arthritis paw volume (ml)

Groups	Days				
	0	4	8	14	21
Normal	0.81 ± 0.04	0.82 ± 0.04	0.82 ± 0.05	0.82 ± 0.03	0.83 ± 0.04
Control	0.92 ± 0.05	1.90± 0.17##	2.84 ± 0.10##	3.03 ± 0.12##	2.97 ± 0.10##
Diclofenac10mg/kg	0.91 ± 0.03	1.53± 0.21	1.91± 0.30**	1.57± .15**	1.33± 0.11**
AEAM 100mg/kg	0.90 ± 0.04	1.80 ± 0.10	2.48 ± 0.11	2.61 ± 0.20	2.45 ± 0.12
AEAM 200mg/kg	0.88 ± 0.04	1.66 ± 0.13	2.27 ± 0.12	2.31 ± 0.17*	2.20 ± 0.24**
AEAM 400mg/kg	0.93 ± 0.04	1.64 ± 0.13	1.97 ± 0.25**	2.15± 0.28**	1.55± 0.17**

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01. as compared with control (One-way ANOVA followed by Dunnet's test). ## indicates significant induction when compared with normal group.

Table 2: Effect of AEAM on % inhibition of CFA induced paw volume.

Groups	% Inhibition of paw volume.				
	Day 0	Day 4	Day8	Day 14	Day 21
Control	-	-	-	-	-
Diclofenac 10mg/kg	-	37.11%	47.91%	69.04%	79.90%
AEAM 100mg/kg	-	7.21%	15.62%	17.70%	24.01%
AEAM 200mg/kg	-	19.58%	27.60%	31.90%	35.29%
AEAM 400mg/kg	-	26.80%	37.50%	49.04%	69.60%

Table 3: Effect of AEAM on CFA induced arthritis paw diameter (mm)

Groups	Days				
	0	4	8	14	21
Normal	8.83± 0.30	9.00± 0.36	8.83± 0.47	8.83± 0.54	9.16± 0.60
Control	9.00± 0.44	18.33± 1.62##	25.00± 1.06##	23.17± 0.94##	23.00± 1.50##
Diclofenac10mg/kg	10.00± 0.51	15.50± 1.23	17.50± 0.42**	14.83± 0.79**	13.67± 0.80**
AEAM 100mg/kg	9.00± 0.44	17.50± 1.76	22.17± 0.94	20.00± 1.41	19.33± 0.88
AEAM 200mg/kg	8.00± 0.25	16.17± 1.40	20.50± 0.84**	18.17± 1.13**	16.17± 0.70**
AEAM400mg/kg	9.00± 0.44	15.67± 0.84	19.67± 1.22**	17.83± 0.98**	13.17± 1.01**

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01. as compared with control (One-way ANOVA followed by Dunnet's test). ## indicates significant induction when compared with normal group

Table 4: Effect of AEAM on various Biochemical parameters.

Groups	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total Protein (gm/dl)
Normal	37.23± 4.68	52.91± 7.05	118.1± 6.9	8.72± 0.57
Control	63.13± 1.64##	111.7± 11.85##	277.5± 25.95##	3.79± 0.56##
Diclofenac 10mg/kg	42.88± 2.54**	56.57± 7.54**	120.9± 23.92**	8.03± 0.93**
AEAM 100mg/kg	54.89± 2.94	137.8± 10.12	218.6± 4.39	5.41± 0.95
AEAM 200mg/kg	47.03± 6.73*	81.00± 11.48	190.1± 32.31*	7.21.± 0.81*
AEAM 400mg/kg	45.08± 3.53*	72.09± 7.73*	159.1± 16.97**	7.92± 0.97**

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01. as compared with control (One-way ANOVA followed by Dunnet's test). ## indicates significant induction when compared with normal group.

Table 5: Effect of AEAM on Body wt.

Group	Mean Body wt (gm)		Mean changes in body wt
	Day 0	Day 21	
Normal	153.30 ± 5.7	195.10 ± 4.8	41.80 ± 0.90
Control	162.60 ± 8.9	176.80 ± 7.6	14.20 ± 1.3##
Diclofenac10mg/kg	157.10 ± 4.8	196.50 ± 5.40	39.40 ± 0.60**
AEAM 100mg/kg	161 ± 12.9	174.90 ± 10.8	13.90 ± 2.1
AEAM 200mg/kg	163 ± 14.5	190.40±11.9	27.40 ± 2.6*
AEAM 400mg/kg	159.60 ± 7.8	196.40±11.1	36.80 ± 3.3**

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01. as compared with control followed by Student's-t test. ## indicates significant induction when compared with normal group.

DISCUSSION

Complete Freund's adjuvant (CFA) induced arthritis is the most widely used chronic test model in which the clinical and pathological changes are comparable with those seen in human rheumatoid arthritis [14]. In CFA induced arthritis bacterial peptidoglycan and muramyl dipeptide are responsible for its induction. It occurs through cell mediated-autoimmunity by structural mimicry between mycobacteria and cartilage proteoglycans in rats [15].Earlier findings suggests that changes in paw volume and paw diameter have been found to be associated with activation of macrophages by CFA results in the production of several cytokines including IL-1, IL-6, interferon-γ (IFN-γ) and TNF-α that have been implicated in immune arthritis which are responsible for changes in paw swelling and paw diameter [16]. In the present investigation prophylactic administration of AEAM significantly suppressed the paw swelling and paw diameter in both acute and chronic phase which may be due to the suppression of different cytokines such as IL-1, IL-6, interferon-γ (IFN-γ) and TNF-α which are responsible for changes in paw swelling and paw diameter.

Assessment of the serum levels of SGOT, SGPT and ALP provides an excellent and simple tool to measure the anti-arthritic activity of the target drug. Earlier findings suggest that CFA administration in rats immunologically alters the hepatic biochemistry which are responsible for changes in the level of Serum SGOT, SGPT and ALP. Serum SGOT and SGPT have been reported to play a vital role in the formation of biologically active chemical mediators such as bradykinins in inflammatory process. Elevated levels of serum ALP in CFA induced arthritic rats can be due to localized bone loss in the form of bone erosion and peri-articular osteopenia. In the present study, the challenge with CFA was significantly elevated the serum SGOT, SGPT and ALP levels. However prophylactic administration AEAM showed significant changes in the SGOT, SGPT ALP levels. This

effect may be related to the significant immunological alteration in the hepatic biochemistry by AEAM.

Changes in the body weight have also been used to assess the course of the disease and the response to the drug therapy. As the incidence and severity of arthritis increased, the changes in the body weights of the rats also occurred during the course of the experimental period. Earlier findings suggest that changes in body weight during arthritic inflammation are due to deficient absorption of nutrients through the intestine and distress caused by the severity of the arthritis [17]. In present study the evidence of restoration of the body weight in rats treated with the AEAM may involve the improvement of intestinal absorption of the nutrients and the reduction in the distress caused by the severity of the arthritis.

CONCLUSION

On the basis of the results obtained in this study we conclude, and propose that possibly, the potent anti-arthritic effect of *Aegle marmelos* leaves extract. Earlier findings suggest that protective role of alkaloids and flavonoids in the treatment of arthritis because they are responsible for inhibition of inflammatory mediators such as IL-1, TNF-alpha, Prostaglandins (PGs) which are responsible for development of arthritis and its complications. In present study phytochemical investigation of AEAM showed that presence of alkaloids and flavonoids. So, anti-arthritic activity of the AEAM may be due to the presence of alkaloids and flavonoids. The study also demonstrates not only its ability in overcoming arthritis and its complications (bone marrow depression, splenomegaly, anemia) but also clinical signs as evidenced in paw edema and paw diameter. Improvement in health parameter such as body weight indicating its beneficial effects while recovery from arthritis. The findings from the present study justify the traditional use of the plant in Indian ayurvedic medicine in the treatment of arthritis. However, further fractionation and isolation of extract is required to observe safety, efficacy and potency of *Aegle marmelos* against arthritis.

REFERENCES

1. *Feghali C., Wright T., Cytokines in acute and chronic inflammation. Journal of Rheumatology and Clinical Immunology 1997; 2:3: 12-26.*
2. *Chris D., Meletis N., Rheumatoid Arthritis: Etiology and Naturopathic Treatments. Alternative & complementary therapies 2001; 3:2: 348-54.*
3. *Ehab S., Desoky E., Pharmacotherapy of Rheumatoid Arthritis: An Overview. Current Therapeutic Research 2001; 62: 92-112.*
4. *Rajendaran R., Krishnakumar E., Anti-arthritic activity of Premna serratifolia linn. Wood against adjuvant induced arthritis. Avicenna Journal of Medicine 2010; 2:2: 101-06.*
5. *Tripathy S., Sahoo S., Pradhan D., Evaluation of anti arthritic potential of Hybanthus enneaspermus. African Journal of Pharmacy and Pharmacology 2011; 3:12: 611-14.*
6. *Chakraborty M., Gupta K., The potential of interaction of methanolic extract of aegle marmelos leaf extract with hydrochlorothiazide against isoproterenol induced myocardial damage in rats. International Journal of Pharmaceutical Sciences Review and Research 2011; 2:1: 33-39.*
7. *Sivraj R., Balakrishnan A., Preliminary phytochemical analysis of Aegle marmelos. International Journal of Pharmaceutical Sciences and Research 2011; 2:1: 146-50.*
8. *Kirtikar K., Basu B., Indian medical plant, 1st ed. Delhi: International book publication, 1995. p.499-502.*
9. *Upadhya S., Kshama K., Study of hypoglycemic and antioxidant activity of Aegle marmelos in alloxan induced diabetic rats. Indian Jounal of Physiolology and Pharmacology 2004; 48:4: 476-80.*
10. *Khandelwal K., Practical pharmacognosy technique and experiments, 2nd ed. Mumbai: Nirali prakashan; 2006. p.162-65.*
11. *OECD guideline for testing of chemicals. Acute Oral Toxicity Method No. 423. CPCSEA guidelines, Section 15 of the Prevention of Cruelty to Animals Act, 1960, Ministry of environment and forest (AWD), Government of India 2001.*

12. *Ramprasath V., Shanthi P., Anti-inflammatory effect of Samecarpus Anacardium linn. nut extract in acute and chronic inflammatory conditions. Pharma Bulletine 2004; 12:2: 2028-31.*
13. *Woode E., Ainoonson G., Anti-arthritic and antioxidant properties of the ethanolic stem bark extract of Newbouldia laevis Seaman ex Bureau. Journal of Medicinal Plants Research 2008; 2:8: 180-88.*
14. *Bansod M., Kagathara V., Evaluation of analgesics and anti inflammatory activity of a poly-herbal formulation. International Journal of Pharmaceutical and Technical Research 2010; 2:15: 20-27.*
15. *Shete R., Upasani C., Adak V., Evaluation of anti-inflammatory and anti-arthritic activities of ethanolic extract of Vernonia anthelmintica seeds. Journal of Cell and Tissue Research 2010; 10:5: 69-80.*
16. *Bhardwaj L., Patil K., Study on efficacy of treatment with Ficus benghalensis leaf extracts on freunds adjuvant induced arthritis in rats. International Journal of Drug Development & Research 2010; 2:7: 44-49.*
17. *Eric G., Lawrence J., Rheumatoid Arthritis and its therapy: The text book of therapeutics drug and disease management, 16th ed. Delhi: Williams and Wilkins publications; 1996. p.579-590*