

## RESEARCH ARTICLE

## Preparation of Fast Dissolving Tablet by Some Traditionally Used Medicinal Plants &amp; Its Anti-arthritic Evaluation in FCA Induced Arthritis

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## ABSTRACT

**Aim-** The aim of the study was to evaluate the anti-arthritic activity of fast dissolving tablet prepared by methanolic extract of some traditionally used medicinal plants for arthritis. **Material & Methods-** Fast dissolving tablet of methanolic extract of selected medicinal plants were prepared by using Crospovidone &  $\beta$ -cyclodextrin by kneading methods. F1 formulation was evaluated in FCA induced arthritis and various lysosomal enzymes i.e. Cathepsin-D,  $\beta$ -glucuronidase and  $\beta$ -N-acetyl glucosaminidase were estimated. **Results-** Treatment by F1 formulation showed a significant decrease in all lysosomal enzymes as compared to arthritic control. Prednisolone also showed highly significant effect on lysosomal enzymes when compared to arthritic control. **Conclusion-** The prepared herbal fast dissolving tablets shows good disintegration property and dissolution rate. From the results of the study, we may conclude that F1 formulation might be act on membrane stabilization and membrane stability modulating effect is an important mechanism of anti-arthritic activity.

**Keywords:** Fast Dissolving Tablet, glucuronidase and  $\beta$ -N-acetyl glucosaminidase, Crospovidone, *Nyctanthes arbor-tristis*

## INTRODUCTION

RA is one of many autoimmune diseases that predominate in females. The ratio of female to male patients may vary from 2:1 to 4:1. Pregnancy usually is associated with remission of the disease with subsequent relapses after delivery. Clinically, RA is characterized by Polyarthritic, swelling and, in many cases, manifests extra-articular involvement. In the early stage of the disease, typical signs and symptoms are swelling and pain of the proximal interphalangeal and metacarpophalangeal joints. Later, the larger joints become affected, especially those of the arms, feet and knees. In addition, RA can affect other systems of the body, and this may range from rheumatoid nodules to life-threatening vasculitis (Smolen & Steiner 2003). The present study was designed to formulate & evaluate the fast dissolving tablet of methanolic extract of *Nyctanthes arbor-tristis*, *Alstonia scholaris* and *Butea monosperma* & evaluation of its anti-arthritic activity.

## MATERIAL &amp; METHODS

## Collection and authentication of the plant materials

The leaves of *Nyctanthes arbor-tristis* and *Alstonia scholaris* and roots of *Boerhaavia diffusa* and flowers of *Butea monosperma* were collected from outfield and also purchased from local markets. Plant was identified by the Botanist, Research Officer; Botany (Scientist C) at Central Council for Research in Ayurveda, Govt. of India.

## Preparation of Total Crude Extract

Accurately weighed quantity of leaf powder of *Nyctanthes arbor-tristis* and *Alstonia scholaris*, dried flowers of *Butea monosperma* were defatted by using petroleum ether. The mark were dried, weighed and then again extracted by using methanol by soxhlet apparatus for 72 h. The extract of all plants were dried completely under reduced pressure and finally converted into powder. After drying, the respective extracts were weighed and percentage yield was determined (Mukherjee, 2002).

## Preliminary Phytochemical Tests

Qualitative chemical tests of Methanolic extracts were subjected to various chemical tests to detect various phytoconstituents (Kokate, 2003; Khandelwal, 2006).

### Preparation of fast dissolving tablet

Herbal Fast dissolving tablets were prepared by direct compression method using various formulation additives in varying concentrations. All the ingredients were powdered separately in a clean and dry porcelain mortar and then they were passed through # 60 mesh sieve. The extract and

$\beta$ - cyclodextrin were complexed (kneading method) and then all the additives were mixed thoroughly in an inflated polyethylene pouch in a geometric ratio of their weight. Then the powder mixture was compressed in to the tablets of 500 mg weight. (Patil *et al.*, 2011).

**Table No 1-Formulation of Fast Dissolving Tablets of Methanolic Extracts (Formula as per 500 mg)**

Ingredients (mg/tablet)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Extract of <i>Nyctanthes arbor-tristis</i>	100	100	100	100	100	100	100	100	100
Extract of <i>Alstonia scholaris</i>	100	100	100	100	100	100	100	100	100
Extract of <i>Butea monosperma</i>	100	100	100	100	100	100	100	100	100
$\beta$ -cyclo dextrin	100	100	100	100	100	100	100	100	100
Crospovidone	15	-----	-----	15	-----	-----	15	-----	-----
Sodium starch glycolate	-----	20	-----	-----	20	-----	-----	20	-----
Mixture of CP + SSG	-----	-----	25	-----	-----	25	-----	-----	25
MCC	65	60	55	65	60	55	65	60	55
Sodium saccharin	10	10	10	10	10	10	10	10	10
Mg.sterate	5	5	5	5	5	5	5	5	5
Talc	5	5	5	5	5	5	5	5	5
<b>Total</b>	<b>500</b>								

### ACUTE TOXICITY STUDY OF FORMULATION F1

Acute toxicity study was carried out as per Miller and Tainter (graphical) method.

### ANTI-ARTHRITIC EVALUATION OF FORMULATION F1 IN FCA INDUCED ARTHRITIS IN RATS

The arthritis was induced by injection of 0.1 ml of FCA in all the groups except normal control in sub plantar region of right hind paw in rat. The treatment of formulation F1 (50 & 100 mg/kg) was started once daily from day 14<sup>th</sup> to 28<sup>th</sup> from the day of adjuvant injection. The paw volume is measured before induction and after treatments at a regular interval (Arulmozhi *et al.*, 2011; Ignacimuthu *et al.*, 2011).

The animals were divided into five groups and each group comprising of six animals.

**Group-I:** Normal control treated with 1% Tween 80 p.o.

**Group-II:** Arthritic control treated with 0.1 ml of FCA in right hind paw of rat

**Group-III:** Treated with formulation F1 50 mg/kg p.o., daily from 14<sup>th</sup> day to 28<sup>th</sup> day

**Group-IV:** Treated with formulation F1 100 mg/kg p.o., daily from 14<sup>th</sup> day to 28<sup>th</sup> day

**Group-V:** Treated with prednisolone 10 mg/kg p.o., daily from 14<sup>th</sup> day to 28<sup>th</sup> day

The animals were sacrificed after collection of blood sample by retro orbital sampling and mixed with EDTA & liver is removed out for estimation of lysosomal enzymes on 29<sup>th</sup> day.

### Measurements of paw volume

Paw volume of all rats were measured by plethysmograph based on mercury displacement methods at a regular interval on 0, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> day (Mali *et al.*, 2013).

### Arthritis assessment

The severity of the arthritis in each paw was quantified daily by a clinical score measurement from 0 to 4 as follows: 0 – no macroscopic signs of arthritis (swelling or erythema), 1 – swelling of one group of joints (namely, wrist or ankle joints), 2 – swelling of two groups of swollen joints, 3– swelling of three groups of swollen joints, 4 – swelling of the entire paw (Arulmozhi *et al.*, 2011).

### Estimation of lysosomal enzymes from liver

Liver homogenates were centrifuged at 600g for 10 min. The sediment which containing nuclei, Unbroken cells and plasma membranes were separated and the supernatant was subjected to centrifugation at 16,000g for 30 min. The sediment was suspended in 0.25M sucrose buffer. Enzyme activity in the supernatant was determined (Walter & Schutt, 1974; Kandaswamy *et al.*, 2007; King, 1965b; Kumar *et al.*, 2009; Arulmozhi *et al.*, 2011).

### Estimation of Cathepsin-D (Cat-D)

0.9 ml of buffered substrate was mixed with 0.1 ml of enzyme preparation and incubated for 2 h at 37<sup>0</sup>C. The reaction was stopped with 1.0 ml of 5% TCA and the samples were centrifuged for 10 min. To the control tubes, the enzyme preparation was added after the addition of TCA. To 1.0 ml

supernatant, 1.0 ml of 5% NaOH and 4.5 ml of alkaline copper reagent were added. After 20 min, 0.5 ml of Folin's phenol reagent was added and the colour developed was read at 640 nm after 10 min. The standards were treated similarly. Enzyme activity is expressed as micromoles of tyrosine liberated per hour per milligram of protein at 37°C (Biber *et al.*, 1981; Ignacimuthu *et al.*, 2011).

### Estimation of $\beta$ -D-Glucuronidase

0.05 ml of substrate, 0.05 ml of acetate buffer, 0.05 ml of homogenate was incubated at 37°C for 1 h. The reaction was arrested by the addition of 3.9 ml of glycine buffer. Standards were also run simultaneously along with a blank. The colour developed was read at 420 nm using a colorimeter. The enzyme activity is expressed as micromoles of p-nitrophenol formed per hour per milligram of protein (Kawai & Anno, 1971; Ignacimuthu *et al.*, 2011).

### Estimation of N-acetyl- $\beta$ -D-glucosaminidase

To 0.2 ml enzyme preparation, 0.1 ml of buffered substrate was added and incubated at 37°C for 40 min. At the end of the incubation period, the reaction was arrested by the addition of 2.2 ml of 0.2M glycine buffer and the colour developed was read at 420 nm using colorimeter. The enzyme activity is expressed as micromoles of p-

nitrophenol formed per hour per milligram of protein (Marhur, 1976; Ignacimuthu *et al.*, 2011).

### STATISTICAL ANALYSIS

The values are expressed in mean  $\pm$  SEM. The results were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's "t" test to determine the statistical significance.  $p < 0.05$  was chosen as the level of significance.

### RESULTS

#### PRELIMINARY PHYTOCHEMICAL SCREENING

The preliminary phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds.

#### ANTI-ARTHRITIC ACTIVITY OF F1 FORMULATION IN FCA INDUCED ARTHRITIS IN RAT

##### Effect of F1 on paw volume in FCA induced arthritis in rat

In the treated groups, paw volume was significantly decreased as compared to arthritic control rats. The paw volume was significantly decreased at 50 and 100 mg/kg.

Table No 2: Effect of F1 Formulation on paw volume in FCA induced arthritis in rats

Groups	Zero Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	21 <sup>st</sup> Day	28 <sup>th</sup> Day
Normal Control	0.29 $\pm$ 0.04	0.30 $\pm$ 0.07	0.31 $\pm$ 0.7	0.31 $\pm$ 0.2	0.31 $\pm$ 0.3
Arthritic Control	0.30 $\pm$ 0.06	0.77 $\pm$ 0.22**	1.73 $\pm$ 0.13***	1.88 $\pm$ 0.12***	1.92 $\pm$ 0.11***
F1 50 mg/kg	0.31 $\pm$ 0.03	0.79 $\pm$ 0.14**	1.71 $\pm$ 0.18***	0.82 $\pm$ 0.14**	0.64 $\pm$ 0.23**
F1 100 mg/kg	0.31 $\pm$ 0.02	0.83 $\pm$ 0.14**	1.68 $\pm$ 0.11***	0.73 $\pm$ 0.19***	0.61 $\pm$ 0.22***
Prednisolone 10 mg/kg	0.32 $\pm$ 0.41	0.88 $\pm$ 0.21**	1.68 $\pm$ 0.22***	0.71 $\pm$ 0.14***	0.51 $\pm$ 0.20***

Values are expressed as mean $\pm$ SEM,  $n = 6$  in each group; \*\* $p < 0.01$ , compared to arthritic control. \*\*\*  $p < 0.001$ , compared to arthritic control.

#### Effects of F1 Formulation on arthritic scores

The sign of arthritis appeared from 20–22 days after immunization and reached its maximum level at 45<sup>th</sup> day. The macroscopic sign of severe arthritis at 45<sup>th</sup> day included swelling, redness deformity and ankylosis in hind paw and ankle joints. The symptoms of arthritic control rats showed significant difference as compared to the hind paw of normal rats. Such symptoms were, however, found to be very less in the forelimbs. Whereas F1 (50 mg/kg) treated arthritic rats showed redness and swelling only with moderate arthritis, the arthritic rats treated with F1 (100 mg/kg), however, showed almost no sign of arthritis and appeared essentially similar to normal rats.

Table No 3: Effect of F1 on poly-arthritis index in FCA induced arthritis in rats

Groups	7 <sup>th</sup> Day	14 <sup>th</sup> Day	28 <sup>th</sup> Day
Arthritic Control	3.42 $\pm$ 0.13	3.92 $\pm$ 0.21	4.11 $\pm$ 0.12
F1 50 mg/kg	3.31 $\pm$ 0.21	3.79 $\pm$ 0.22	1.96 $\pm$ 0.23***
F1 100 mg/kg	3.34 $\pm$ 0.22	3.92 $\pm$ 0.21	1.63 $\pm$ 0.27***
Prednisolone 10 mg/kg	3.34 $\pm$ 0.23	3.92 $\pm$ 0.14	1.52 $\pm$ 0.11***

Values are expressed as mean $\pm$ SEM,  $n = 6$  in each group; \*\*\*  $p < 0.001$ , compared to arthritic control.

#### Effects of F1 on lysosomal enzymes in arthritic and normal rats

There is highly significant decrease in N-Acetyl- $\beta$ -D-glucosamine &  $\beta$ -D-Glucuronidase levels in F1 treated groups as compared to arthritic rats. Prednisolone shown a highly significant decrease in N-Acetyl- $\beta$ -D-glucosamine &  $\beta$ -D

Glucuronidase and moderately decrease in Cathepsin-D & Acid Phosphatase levels.

**Table No 4: Effect of F1 on lysosomal enzymes in control and arthritic rats**

Groups	Cathepsin-D	N-Acetyl-β-D-glucosamine	β-D-Glucuronidase
Normal Control	0.31±0.02	31.62±1.40	32.81±1.16
Arthritic Control	0.90±0.06**	51.12±3.18**	52.02±1.52**
F1 50 mg/kg	0.72±0.03*	42.17±1.24*	48.81±1.27**
F1 100 mg/kg	0.42±0.12**	38.12±2.10***	40.26±1.89***
Prednisolone 10 mg/kg	0.36±0.11**	35.20±2.12***	37.19±1.69***

Values are expressed as mean±SEM, n = 6 in each group; \*\*p<0.01, compared to arthritic control.

\*\*\* p<0.001, compared to arthritic control.

## DISCUSSION

Recently, macrophage migration inhibitory factor (MIF) has been considered to have pro-inflammatory effects in RA. MIF is implicated in leukocyte recruitment, activation, proliferation and survival, as well as the production of pro-inflammatory cytokines and mediators, and mechanisms of bone and cartilage injury, all of which contribute to the pathology of RA (Eric *et al.*, 2006). Macrophages are one of the key players in most of the chronic inflammatory diseases (Allison and Davies, 1974) like arthritis and act as an important link in development of chronic inflammation through persistence of acute inflammation. The micromeritic properties were determined for all the physical mixtures of *Nyctanthes arbor-tristis*, *Alstonia scholaris* and *Butea monosperma*. Various evaluation parameters were used to evaluate the different formulation and from the results (data not shown here) we found that formulation F1 was found to be very satisfactory. The drug content was found to be close to 100% in F1 formulations.

F1 formulation which contains 3% super disintegrates releases 96.01%, drug respectively at the end of 60 min. The rapid drug dissolution might be due to easy breakdown of particles and rapid absorption of drug into the dissolution medium. CFA-induced experimental model for arthritis is considered closest to simulating human rheumatoid arthritis and therefore it is the most widely used chronic test model in which the associated clinical and Histopathological changes are comparable to those seen in human form (Billingham & Davies, 1979; Butler *et al.*, 1992).

Anti-arthritis activity of prepared F1 formulation was performed in FCA induced arthritis in rats as described preliminary activity. Effect of F1

formulation was seen on paw volume, Polyarthritic index and various lysosomal enzymes like Cathepsin-D, N-Acetyl-β-D-glucosamine & β-D-Glucuronidase. F1 formulation had significant decreasing effect on paw volume, Polyarthritic index as compared to arthritic animals. It is postulated that lysosomal mechanism such as hydrolytic enzymes or cationic proteins play important roles in the beginning of inflammation, tissue injury and connective tissue breakdown (Weissman and his co-workers, 1964, 1969; Shen, 1967, Janoff and Zweifach, 1964). Anderson (1970) found higher levels of catalytic enzymes in inflamed tissue or serum of arthritic rats as compared to normal animals. In rat adjuvant arthritis, it has been stressed that the potential destructive capacity of connective tissue is acid hydrolases and is liberated within the endogenous cellular elements of connective tissue or derived from migrating leukocytes (Anderson, 1970).

Cathepsin-D, β-glucuronidase and β-N-acetyl glucosaminidase are such lysosomal glycohydrolases that are mainly responsible for the opening up of collagen fibers for the attack by collagenolytic enzymes. Since the degradation of collagen is mainly associated with the collagenolytic enzymes, studies were undertaken to determine the activities of certain proteolytic enzymes particularly those involved in the degradation of collagen and other connective tissue components in the liver of adjuvant arthritic rats (Ekambaram *et al.*, 2011).

## CONCLUSION

The proposed mechanism of formulation may be that, there was increase in the lysosomal enzymes of liver and serum of arthritic animals when compared with normal rats. Increased activities of these enzymes show increased fragility of cells and injury during inflammation. The marked decreases in lysosomal enzymes were seen F1 treated animals. From the results of the study, we may conclude that F1 formulation might be act on membrane stabilization and membrane stability modulating effect is an important mechanism of anti-arthritis activity.

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