



## Comparative Anti-Arthritic Study of *Cassia fistula* with Naproxen in Rheumatoid Arthritis Murine Model

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### ABSTRACT

**Introduction:** Rheumatoid arthritis is a chronic inflammatory autoimmune disease causing redness, warmth, swelling and immobility of the affected joints. Naproxen, a non-steroidal anti-inflammatory drug (NSAID), is used for the treatment of RA but has many adverse effects. Many plants have anti-inflammatory activity and can treat arthritis without any side effects. *Cassia fistula* could have anti-arthritic and lower toxic potential. **Aims and Objective:** Estimation of anti-arthritic aspect of *Cassia fistula* (Amaltas) and its comparison with Naproxen in Complete Freund's Adjuvant (CFA) induced murine RA model. **Place and Duration of Study:** This study was conducted in UVAS, in batches of 15 days for 4 months. **Material and Methods:** This murine experimental study was conducted for 15 days on 56 male albino rats divided into seven groups of eight rats each. Group 1 was treated as negative control while group 2 (positive control), group 3-7 (therapeutic groups) were given CFA injection (0.2ml) on day 1 followed by naproxen (25mg/kg), anthraquinone extract (250mg/kg and 500mg/kg) and methanolic extract (250mg/kg and 500mg/kg) orally BD on 3 days; 9, 10 and 11 of the study as per the group designation. Caliper measurement of right ankle joint and RA factor (qualitative) was used to evaluate the anti-arthritic effect of *Cassia fistula* on days 1, 9 and 15. **Results:** The ankle swelling and presence of RA factor were significantly reduced in the therapeutic groups as compared to that of the diseased control in a dose dependent manner (500>250) for both methanolic and anthraquinone extracts but less than the standard drug naproxen. **Conclusion:** Although less potent, *Cassia fistula* is a safer alternative to naproxen for treating RA.

**Key words:** *Cassia fistula*, anthraquinone, NSAID, CFA, RA factor.

### INTRODUCTION

Rheumatoid Arthritis (RA), a chronic, increasingly debilitating, autoimmune disorder causes inflammation of one or more small joints, morning stiffness, lethargy and multiorgan dysfunction<sup>1</sup>. It can cause deformity and immobility of joints of fingers, hands, feet and knees<sup>2</sup>. It affects 0.3% to 1% people globally during the most productive period of adulthood between the ages of 20 and 40 years<sup>3</sup>. Early diagnosis and aggressive treatment can improve the disease prognosis.

The goals of treatment are to minimize pain and swelling and to prevent bone deformity and can be achieved by using NSAIDs and disease modifying

antirheumatic drugs (DMARDs). NSAIDs are the most commonly used drugs worldwide of which ibuprofen and naproxen are mostly used to relieve pain and swelling<sup>4</sup>. Currently more than 10 million prescriptions for naproxen are filled yearly. Naproxen decreases pro-inflammatory prostaglandins; important mediators of inflammation and pain by inhibiting tissue cyclo-oxygenases<sup>5</sup>. Thus, alleviates symptoms of RA but is associated with hepatotoxicity, nephrotoxicity and gastro-intestinal disturbances<sup>6,7,8</sup>.

To avoid these adverse effects safer alternatives derived from botanical sources have started being investigated as sources of new phyto-pharmaceuticals for the treatment of rheumatoid arthritis<sup>4</sup>.

*Cassia fistula* known as Amaltas, is a source of various herbal medicines. Every part of this plant is recognized for its medicinal properties but most importantly the fruit pulp has shown useful application in gout and rheumatism and possesses anti-inflammatory activity<sup>9</sup>. The plant is rich in phenolic antioxidants such as anthraquinone, which is the common active principle in all parts of the *Cassia fistula* plant<sup>10</sup>.

The motivation of study came from the fact that patients of rheumatoid arthritis needed a safer, side effect free new anti-arthritic drug. Hence, the current research was conducted to evaluate and compare the therapeutic anti-arthritic aspect of *Cassia fistula* with naproxen in CFA induced murine model of rats.

This research was unique as it employed the CFA murine model of rheumatoid arthritis for comparison of anti-arthritic potential of the standard drug naproxen and *Cassia fistula* methanolic fruit pulp extract and anthraquinone. Whereas previously carrageenan model had been used and comparison of anti-inflammatory activity of aqueous and alcoholic extracts of cassia fistula with diclofenac was done.

## MATERIAL AND METHODS

This experimental study was conducted in University of Veterinary and Animal Sciences (UVAS) Lahore, after approval from Institutional Review Board (IRB) and was completed in batches of 15 days in a period of 4 months.

Fruit pulp and bark of *Cassia fistula* was collected in the month of April from Botany department of University of the Punjab, Lahore. It was identified and verified by a botanist in PCSIR, Lahore.

### **Preparation of *Cassia fistula* extracts:**

Anthraquinone and methanolic extracts of *Cassia fistula* were made in Applied Chemistry Research Centre, PCSIR Laboratories, Lahore. Fruit pulp and bark of *Cassia fistula* was dried and finely powdered. Extraction was done by the method described below<sup>11</sup>. The extract was used after the confirmatory anthraquinone test.

### **Extraction of anthraquinone:**

30gm powdered *Cassia* fruit pulp was extracted with 150ml ethanol (1:5) using a Soxhlet apparatus which was then heated continuously for 24hrs at solvent's boiling point over a heating mantle.

Anthraquinone (rhein) extract was concentrated, dried and stored with desiccator.

### **Test for Anthraquinones:**

10ml of 1% HCl was added to powdered *Cassia fistula* fruit pulp extract and boiled for 5 minutes. The sample was filtered and allowed to cool. Partition of the cool filtrate was done twice using equal volumes of chloroform and 10% ammonia and then the layer was allowed to separate. Rose pink color indicated the presence of combined anthraquinones<sup>11</sup>

### **Preparation of methanolic extract**

Powdered *Cassia* bark was defatted with petroleum ether (60-80°C) and successively extracted with methanol and double distilled water using soxhlet extractor. The methanolic extract (light brown in color) was dried under reduced pressure using a rotary vacuum evaporator and was refrigerated for further use. The percentage yield was 9% w/w for methanol extract (CFM).

### **Preparation and assessment of rat model of rheumatoid arthritis:**

Arthritis was induced in right hind paw foot pad of each rat using a single dose (0.2 ml) of CFA which contains killed *Mycobacterium tuberculosis* and non-metabolizable oils and is designed to provide continuous release of antigens necessary to stimulate a strong persistent immune response<sup>12</sup>. Resultantly a swelling was noticed around the injection site within a few hours whereas clinical evidence of arthritis was noted on the 9<sup>th</sup> post CFA injection day. The swelling grew gradually over a 15 day period and was associated with declining rat mobility as the arthritis progressed. Treatment was initiated on day 9<sup>13</sup>.

### **Experimental Setup:**

A total of 56 adult male wistar albino rats weighing 170–200 gm were placed in animal house of UVAS Lahore. They were acclimatized for a week and maintained in polypropylene cages at 25 ± 2° C, with relative humidity 45-55% under 12h light and dark cycles and fed with standard laboratory diet with water ad libitum.

They were divided into seven groups with eight rats in each. Every rat of each group was clearly and carefully numbered. The test extracts and the standard drug was administered orally in the form of a suspension in water as per experimental requirement using 1% carboxymethyl cellulose as suspending agent.

**Group 1:** Healthy control group; it was not treated and was given equal quantity of normal saline.

**Group 2:** Experimental control rats were given a single dose of CFA injection 0.2ml in right hind paw foot pad and left for self-recovery<sup>14</sup>.

**Group 3:** This group was given tab. naproxen 25mg/kg orally BD on day 9, 10 and 11<sup>14</sup>.

**Group 4:** Anthraquinone extract 250mg/kg orally BD on day 9, 10 and 11.

**Group 5:** Anthraquinone extract 500mg/kg orally BD on day 9, 10 and 11.

**Group 6:** Methanolic extract 250mg/kg orally BD on day 9, 10 and 11.

**Group 7:** Methanolic extract 500mg/kg orally BD on day 9, 10 and 11.

On day 1 CFA injection was given to induce RA in all groups except group 1.

Caliper measurement of right ankle joint was done and blood sample for RA factor (qualitative) was collected by cardiac puncture on day 1, 9 and 15.

### Statistical Analysis:

Data was analyzed by SPSS version 20.0 and described by using Mean  $\pm$  SD for each group. One-way ANOVA test was used for comparison between groups and p-value < 0.05 was taken as statistically significant.

## RESULTS

	Mean			Standard Deviation		
	Day 1	Day 9	Day 15	Day 1	Day 9	Day 15
Group 1	0.24	0.24	0.24	0.05	0.05	0.05
Group 2	0.58	0.54	0.53	0.05	0.07	0.07
Group 3	0.58	0.30	0.25	0.05	0.00	0.05
Group 4	0.58	0.51	0.50	0.05	0.04	0.05
Group 5	0.58	0.29	0.40	0.05	0.04	0.05
Group 6	0.58	0.55	0.50	0.05	0.05	0.05
Group 7	0.58	0.40	0.30	0.05	0.00	0.05

**Table-1:** Caliper measurement (cm) of right ankle joint of rats given Naproxen and *Cassia fistula* extracts as per group designation (N=8)

The difference among groups was significant with p-value < 0.05.

	Rheumatoid Factor					
	Day 1		Day 9		Day 15	
	Absent	Present	Absent	Present	Absent	Present
Group 1	8 (100%)	-	8 (100%)	0 (0.0%)	8 (100.0%)	0 (0.0%)
Group 2	8 (100%)	-	1 (12.5%)	7 (87.5%)	1 (12.5%)	7 (87.5%)
Group 3	8 (100%)	-	1 (12.5%)	7 (87.5%)	3 (37.5%)	5 (62.5%)
Group 4	8 (100%)	-	1 (12.5%)	7 (87.5%)	3 (37.5%)	5 (62.5%)
Group 5	8 (100%)	-	1 (12.5%)	7 (87.5%)	5 (62.5%)	3 (37.5%)
Group 6	8 (100%)	-	1 (12.5%)	7 (87.5%)	3 (37.5%)	5 (62.5%)
Group 7	8 (100%)	-	1 (12.5%)	7 (87.5%)	5 (62.5%)	3 (37.5%)
p- value			0.000		0.015	

**Table-2:** Presence of Rheumatoid factor in rats given Naproxen and *Cassia fistula* extracts as per group designation (N=8)

Fisher's exact test revealed a significant association between rheumatoid factor and study groups (p-value=0.015). The proportion of animal with rheumatoid factor was higher in groups 2,3,4 and 6.

## DISCUSSION

Rheumatoid arthritis is an autoimmune disease that causes progressive chronic inflammation of joints and other systemic effects. Pathophysiology of RA involves the activation of T cells, B cells and pro-inflammatory cytokines<sup>15</sup>.

Naproxen is a propionic acid derivative related to the arylacetic acid group of non-steroidal anti-inflammatory drugs and is used in the treatment of rheumatoid arthritis which is characterized by persistent synovitis, systemic inflammation, and auto-antibodies (particularly to rheumatoid factor)<sup>16</sup>. Phytotherapy has an important role in treating arthritis<sup>4</sup>. *Cassia Fistula*, Amaltas was previously researched and showed beneficial effects in Carrageenan induced in-vitro murine model of arthritis<sup>17</sup>.

The following parameters were analyzed in detail.

### Rat Ankle Thickness (Caliper measurement):

The arthritic rats exhibited soft tissue swelling and redness around the right ankle joint, 8 days after CFA induction and immobility on 9<sup>th</sup> day, assessed by decreased access of rats towards food and

water<sup>13</sup>. This showed the acute phase of arthritis and was due to edema of joint capsule and ligament which was measured through vernier calipers.

Our results revealed a significant reduction in ankle swelling in the therapeutic groups on day 15 as compared to 1<sup>st</sup> day, in a dose dependent manner (500>250) for both methanolic and anthraquinone extracts of *Cassia fistula* but less than the standard drug naproxen (25mg/kg BD) as shown in table 1.

These results were comparable to a previous study in which carrageenan induced arthritis model was used to evaluate the effect of orally administered extracts of *Cassia fistula* and significant activity of methanolic extract was noted<sup>14</sup>. Similarly, here too the anti-inflammatory effect seen could be due to suppression of inflammatory mediators such as histamine, prostaglandin and serotonin due to inhibitory hydroxyl scavenging activity<sup>18</sup> and an antioxidant effect by inhibiting lipid peroxidation<sup>19</sup>.

#### **Rheumatoid factor:**

Rheumatoid factor is closely linked with the pathology of rheumatoid arthritis. Initially all the rats were normal and rheumatoid factor was absent while it remained absent throughout the experiment in the healthy control group.

At the end of the study on day 15, 87.5% of group 2 rats had rheumatoid factor, while in therapeutic model, group 3, 4 and 6 showed 37.5% lower presence of rheumatoid factor when compared with group 2. While group 5 and 7 exhibited 62.5% lower presence of rheumatoid factor than group 2 as shown in table 2. Overall difference among groups was significant with p-value < 0.05. Reduction in RA factor could be due to anti-inflammatory and anti-oxidant activity of *Cassia fistula*<sup>14,18</sup>.

Our findings on the lowering of rheumatoid factor by methanolic and anthraquinone extract of *Cassia fistula* were non-comparable due to paucity of research on the subject.

#### **CONCLUSION**

This was an innovative research in which *Cassia fistula* (Amaltas) and naproxen were compared in their anti-arthritic aspects in a CFA induced murine model of RA. The CFA model used was distinctive as it greatly simulated clinical RA.

In conclusion, a less efficacious, dose dependent but equipotent anti-arthritic potential of anthraquinone and methanolic extracts of *Cassia fistula* versus naproxen emerged, confirmed by improvement in

the right ankle joint caliper measurement and serum rheumatoid factor levels.

Based upon our research we therefore suggest that *Cassia fistula* has the potential to treat rheumatoid arthritis which is less as compared to naproxen but with a distinct advantage of having none of the known adverse effects of the standard NSAID drugs.

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