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Prophylactic Anti-Arthritic Effect of *Cassia fistula* in Murine Rheumatoid Arthritis Model

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is a major autoimmune disease and an important cause of potentially preventable disability. Many drugs are available for its treatment, however, we need an improved remedy to prevent its onset and worsening in patients afflicted by this disease. Natural substances including plants have been researched for their anti-arthritic potential. *Cassia fistula* could have similar ability. **Aims and Objective**: Evaluation of prophylactic anti-arthritic effect of *Cassia fistula* in Complete Freund's Adjuvant (CFA) induced murine model of RA. **Material and Methods:** The study was carried out for 15 days on 48 male rats divided into 6 groups of 8 rats each. Group 1 (negative control), group 2 (positive control), group 3-6 were given a anthraquinone and methanolic extracts of *Cassia fistula* orally BD on days 1,2 &3, the first dose being given 30min prior to CFA injection (0.2ml). Caliper measurement of right ankle joint and RA factor was used to evaluate the anti-arthritic effect of *Cassia fistula* on days 1, 9 and 15. **Results:** Prophylactic groups showed 36-50% lesser ankle swelling and reduction in rheumatoid factor by 62.5% and 87.5% as compared to that of the diseased control (group 2) in a dose dependent manner (500mg>250mg/kg) for both methanolic and anthraquinone extracts of *Cassia fistula*. **Conclusion:** Overall difference among groups was significant with p-value < 0.05. *Cassia fistula* showed a prophylactic potential in RA treatment due to its anti-inflammatory and anti-oxidant properties.

Keywords: *Cassia fistula,* anthraquinone, CFA, RA factor.

INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic, crippling, debilitating, painful autoimmune disorder which leads to the deformity and immobility of particularly small joints of hands, fingers, knees and feet[1](Fig-1). Its prevalence varies between 0.3% and 1% globally between the ages of 20 and 40 [2].

Cassia fistula commonly known as Amaltas, popularly called "Indian Laburnum" in English has been extensively used in Ayurvedic medicine for various ailments[3] (Fig-2). 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[4].

The plant is rich in phenolic antioxidants such as anthraquinones, flavonoids and flavan-3-ol derivatives, of which anthraquinones and their variants appear to be the common active principle in all parts of the *Cassia fistula* plant[5]. Bark showed the highest antioxidant potential[6]. *Cassia fistula* fruit pulp active principle, has anti-inflammatory ability by inhibiting super oxide anion production from human neutrophils contributing to the overall therapeutic activity of the anthraquinone[7,8].

To investigate these claims further, the current research was conducted, which differed from older researches as it employed the Complete Freund's Adjuvant (CFA) murine model of rheumatoid arthritis to determine prophylactic anti-arthritic effect of anthraquinone derived from *Cassia fistula* bark and fruit pulp. Whereas previously the carrageenan model had been employed.

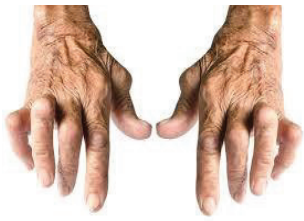

Fig-1: Rheumatoid Arthritis effecting metacarpophalangeal and interphalangeal joint

Fig-2: Cassia fistula (Amaltas)

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 for a period of 3 months.

Fruit pulp and bark of *Cassia fistula* was collected in the month of April from University of the Punjab, Botany Department, 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[9]. The extract was used after the confirmatory anthraquinone test.

Extraction of anthraquinone

30gm powdered cassia fruit pulp + 150ml ethanol (1:5) in Soxhlet apparatus

↓

Heated for 24hrs at solvent's boiling point

↓

Anthraquinone (rhein) extract

↓

Concentrated, dried and stored with desiccator

Preparation of methanolic extract

Powdered cassia bark + petroleum ether

↓

Extraction with methanol and double distilled water in soxhlet extractor

↓

Extract concentrated under reduced pressure in rotary vacuum evaporator

↓

Refrigerated for further use

Test for Anthraquinones

10ml of 1% HCl + fruit pulp extract / bark extract

↓

Boil for 5 minutes

↓

Filter and cool the sample

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[9].

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, provides continuous release of antigens and thus stimulates a strong persistent immune response[10]. Within a few hours a swelling was noticed around the injection site whereas clinical evidence of the arthritis was noted on the 9th post CFA injection day. The swelling grew gradually and was associated with declining rat mobility over a 15day period as the arthritis progressed. Treatment was initiated on day 1 [11].

Assessment of disease progression was made by caliper measurements of ankle joint and RA factor on day 1, 9 and 15.

A total of 48 adult male wistar albino rats weighing 170–200 gm was 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 six groups having eight rats each. Every rat was clearly numbered.

The test extracts were administered in suspension form in water using 1% carboxymethyl cellulose as suspending agent as per experimental requirement:

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 0.2ml dose of CFA injection in right hind paw foot pad sub-cutaneously and left for self-recovery.

Group 3: Anthraquinone extract 250mg/kg orally BD on day 1, 2 and 3.

Group 4: Anthraquinone extract 500mg/kg orally BD on day 1, 2 and 3.

Group 5: Methanolic extract 250mg/kg orally BD on day 1, 2 and 3.

Group 6: Methanolic extract 500mg/kg orally BD on day 1, 2 and 3.

First dose of extracts was given 30min before CFA injection to each group.

On day 1, 9 and 15 caliper measurement of right ankle joint was done and blood samples were collected by cardiac puncture for RA factor (Figs-3,4).

Fig-3: Caliper measurement of ankle thickness

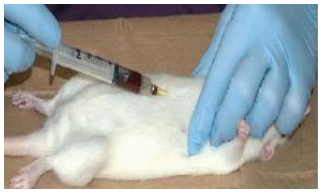

Fig-4: 3ml blood was drawn by cardiac puncture & serum was separated for assessment of RA factor

Statistical Analysis:

Data was analyzed with SPSS version 20.0 and was described by using Mean ± SD for each group. Comparison between groups was done by using one-way ANOVA test and p-value < 0.05 was taken as statistically significant.

RESULTS

	Mean± SD		
	Day 1	**Day 9**	**Day 15**
Group 1	0.24 ±0.05	0.24 ± 0.05	0.24 ± 0.05
Group 2	0.58 ± 0.05	0.54 ± 0.07	0.53 ± 0.07
Group 3	0.50 ± 0.00	0.46 ± 0.05	0.45 ±0.05
Group 4	0.29 ± 0.04	0.26 ± 0.05	0.25 ± 0.05
Group 5	0.55 ± 0.05	0.54 ± 0.05	0.50 ± 0.08
Group 6	0.40 ± 0.00	0.35 ± 0.05	0.34 ± 0.05

Table-1: Caliper measurement of right ankle joint (cm)

The difference among groups was significant with p-value 0.000.

	Rheumatoid factor					
	Day 1		**Day 9**		**Day 15**	
	A	**P**	**A**	**P**	**A**	**P**
Group 1	8 (100%)	-	8 (100%)	-	8 (100%)	-
Group 2	8 (100%)	-	1 (12.5%)	7 (87.5%)	1 (12.5%)	7 (87.5%)
Group 3	8 (100%)	-	4 (50%)	4 (50%)	5 (62.5%)	3 (37.5%)
Group 4	8 (100%)	-	6 (75%)	2 (25%)	7 (87.5%)	1 (12.5%)
Group 5	8 (100%)	-	4 (50%)	4 (50%)	5 (62.5%)	3 (37.5%)
Group 6	8 (100%)	-	6 (75%)	2 (25%)	7 (87.5%)	1 (12.5%)
p-value			**0.011**		**0.005**	

Table-2: Comparison of Rheumatoid factor

A: Absent **P: Present**

Fisher's exact test revealed that there was significant association between rheumatoid factor and study groups (p-value = 0.011). The proportion of animal with rheumatoid factor was higher in groups 2, 3 and 5.

DISCUSSION

Rheumatoid arthritis is a progressive inflammatory autoimmune disease with articular and systemic effects. T cells, B cells and pro-inflammatory cytokines play key roles in the pathophysiology of rheumatoid arthritis[12].

Phytotherapy has been recognized as valuable and readily resource for primary health care and WHO has endorsed its safe and effective use. It is estimated that 80% of the population living in the developing countries rely exclusively on traditional medicine for their primary health care needs[13]. Plants have a great importance in treating arthritis. *Cassia fistula*, Amaltas an indigenous plant was previously researched and showed beneficial effects in Carrageenan induced in-vitro murine model of arthritis[14]. Since prevention is better than cure, patients need a drug that can either prevent the disease or reduce its morbidity.

Bearing this in mind the present research work was conducted on *Cassia fistula* bark (methanolic extract) and fruit pulp (anthraquinone) for preventing rheumatoid arthritis which was generated in-vitro using the CFA model in rats.

The following parameters were analyzed in detail.

Rat Ankle Thickness (Caliper measurement):
In the present investigation the arthritic rats showed a soft tissue redness, warmth and swelling 8 days after CFA induction around the right ankle joint and immobility on day 9. This reflected the acute phase of arthritis and was due to edema of tissues such as ligaments and joint capsules which was measured through vernier calipers.

On day 1 pre-treatment with anthraquinone and methanolic extract in doses of 500mg/kg BD (groups 4 &6) before induction of rheumatoid arthritis and subsequently for the next two days as the disease was developing resulted in a 36-50% lesser ankle swelling in the affected ankle as compared to the that of the diseased control (group 2). Later on, caliper measurements of ankle thickness showed similar changes. Our results revealed a significant reduction on day 9 and 15 in a dose dependent manner (500mg>250mg/kg) for both methanolic and anthraquinone extracts of *Cassia fistula* as shown in Table-1.

These results concurred with a previous study in which carrageenan induced arthritis model was used to evaluate the effect of orally administered extracts of *Cassia fistula* and significant ameliorative activity of methanolic extract was noted[15]. Similarly, here too the reduction in inflammation seen could be due to inhibition of mediators of inflammation such as histamine, serotonin and prostaglandin due to inhibitory hydroxyl scavenging activity[16] and an antioxidant effect by inhibiting lipid peroxidation[17].

Rheumatoid factor:
Rheumatoid factor is intimately 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 group 3 and 5 62.5% of the rats showed a reduction in rheumatoid factor after administration of 250mg/kg BD of anthraquinone and methanolic extracts of *Cassia fistula* respectively.
However, dose dependent effect was seen in groups 4 and 6 which were administered 500mg/kg BD doses of anthraquinone and methanolic extracts, which gave much better results and presence of rheumatoid factor was 87.5% lower than that of group 2. This action could be due to inhibition of inflammatory mediators and lipid peroxidation by the *Cassia fistula* extracts[16,17]. Overall difference among groups was significant with p-value < 0.05.

Our findings on the lowering of rheumatoid factor using methanolic extract and rhein could not be substantiated as no similar study existed to support or refute them.

CONCLUSION

This was a novel research which showed prophylactic anti-arthritic effect of *Cassia fistula* (Amaltas) in a CFA induced murine model of rheumatoid arthritis. The CFA model used was unique as well, as it allowed to develop a form of rheumatoid arthritis which greatly mimicked clinical rheumatoid arthritis.

In conclusion, an extremely interesting, dose dependent prophylactic anti-arthritic potential of anthraquinone and methanolic extracts of *Cassia fistula* 250mg/kg BD and 500mg/kg BD emerged. Corroborating this aspect was a demonstrable improvement in the right ankle joint caliper measurement and serum rheumatoid factor levels.

Based upon our research it can be deduced that prophylactic administration of 500mg/kg BD doses of anthraquinone and methanolic extracts revealed greater efficacy in preventing rheumatoid arthritis in a CFA model. We therefore suggest that *Cassia fistula* has the potential to prevent rheumatoid arthritis and prophylaxis reduces the morbidity of the disease to a great extent.

The CFA Model used simulated the clinical picture of RA to a large extent but did not replicate it exactly. Further research can be done regarding the use of this herb for treating the complications of arthritis for example twisted joints, and deformity.

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