Original Research Article

Effect of Turmeric Extract and Fenugreek Seed Extract on Complete Freund's Adjuvant Induced Arthritis in Rats

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Abstract

Introduction: Arthritis is a joint disorder characterized by inflammation and pain in one or more joints. Complete Freund's adjuvant (CFA) induced arthritis in rats mimic rheumatoid arthritis (RA). Drugs used for treatmentof RA are non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti-rheumatic drugs (DMARDs), glucocorticoids but due to their side effects and high cost, alternative and safer medication is being searched. **Purpose:**To evaluate anti-arthritic effect of Turmeric extract (T. Ex.), Fenugreek seed extract (FSE) on CFA induced arthritis in Wistar rats and compare its effects with standard drugs indomethacin, cyclophosphamide. **Methodology:**Study was conducted at the Department of Pharmacology, King George's Medical University, Lucknow, after ethical approval. 60 Wistar rats were used in this study. On day '0', 0.1 ml CFA was given intradermally in right hind paw, arthritis was induced on day 5. Test drugs used were T. ex. 100 mg/kg, FSE 400 mg/kg and compared with standard drug Indomethacin 3 mg/kg in primary lesion groups (day 5 to day 15), Cyclophosphamide 7 mg/kg in secondary lesion groups (day 12 to day 22). Parameters accessed were, body weight, paw volume, ankle diameter, serum TNF-α, Arthritic index.**Results:**Statistically significant difference was not observed between all treatment groups so turmeric extract and fenugreek seed extract have anti-arthritic effect comparable to indomethacin on primary lesions and cyclophosphamide on secondary lesions.**Conclusion:**Turmeric extract 100 mg/kg, Fenugreek seed extract 400 mg/kg might have a potential usefulness as an alternative to conventional therapy in the management of rheumatoid arthritis.

Key words: Rheumatoid arthritis, Complete Freund's adjuvant, Turmeric extract, Fenugreek seed extract, Wistar Rats

Introduction

Rheumatoid Arthritis (RA) isa joint disorder, characterized by inflammation and pain. RA can affect one or more joints. It causes joints inflammation including synovial membrane (synovitis), often involvement of cartilage and bone. RA can cause loss of motion and in severe cases it can leads to disability. People of all age groups (including children) can be affected. Nearly two-thirds of the people are less than 65 year of age. Prevalenceof RA varies between 0.3% to 1% worldwide, [1,2], 0.75% in India.[3]RA can affect any joint, but small joints in the hands and feet involves more commonly. Sometimes it can affect organs as well, like heart, eyes or lungs. The medications used for the treatment of RA are: Nonsteroidal anti-inflammatory drugs (NSAIDs), Glucocorticoids, Conventional and biological disease-modifying anti-rheumatic drugs (DMARDs). However, due to their side effects, newer and safer mode of treatment is continually being searched.Many drugs that are derived from herbs /plants are also found to be effective in the treatment of the RA on which studies

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has been done like Curcuma longa, Boswellia serrata, Withania somniferum, Zingiber officinale, Garlic, Fish oil, Tripterygium wilfodii.Curcuma longa generally known as turmeric have been used from long time and ascribed for its wound healing, anti-inflammatory property. In current traditional Indian medicine, it is used for the treatment of rheumatism, biliary disorders, cough, anorexia, sinusitis, diabetic wounds, and hepatic disorders.Curcumininhibits autoimmune diseases by regulating inflammatory cytokines such as IL-1 beta, IL-6, IL-12, TNF-alpha and IFN gamma and associated JAK-STAT signalling pathways in immune cells[4]. Curcuminoids are approved by the FDA and recognise as safe [5]. Trigonella foenum graecum or fenugreek is an annual plant, seed extract shows hypoglycemic, hypo-cholesterolemic, antioxidant, immunomodulatory effect. *T. foenumgraecum* is a medicinal plant examined for its anti-inflammatory, antipyretic and antioxidant activities [6]. The effectiveness of *Trigonella foenum graecum*in rheumatoid arthritis may be due in part to its anti-inflammatory and anti-oxidant properties. Ethanolic extract of *T. foenumgraecum* was tested against complete freund's adjuvant-induced arthritis in rats. Administration of *Trigonella foenum graecum* (200 and 400 mg/kg) significantly ($P<0.05$) decreased the paw edema and restored body weight [7]. Complete Freund's adjuvant (CFA) is a reagent frequently used to induce RA in animal models [8]. CFA induced arthritis is a well-established model in rats. It has been extensively used in the study of inflammatory process [9]. Adjuvant-induced arthritis is similar to human RA both in pathological and serological changes [10]. It is validated model to investigate the pathogenesis of RA and autoimmune diseases, to identify potential therapeutic targets [11].

Material and Methods

Study was conducted at the department of Pharmacology, King George's Medical University, Lucknow, after getting approval by Institutional Animal Ethical Committee (IAEC),research project Code Number: 105 / IAEC/ 2018

Experimental Animal

Healthy adult female Wistar rats, having similar physical constitution (in terms of age, body weight), 180-220 gm procured from the IITR, Lucknow (CPCSEA-certified animal house), India after ethical approval. The rats were housed in well ventilated institutional animal house of the King George's Medical University, Lucknow under standard laboratory conditions. The food was given in the form of dry pellets and water to ad libitum. Careand use of animals were done as per CPCSEA guideline.

Materials

I. Complete Freund's adjuvant (CFA)-Arthritis inducing adjuvant, obtained from Sigma Aldrich, USA, administered in a dose of 0.1 ml, intradermally, in right hind paw.

II. Test Drugs

Turmeric- 95% extract,batch no: CAI/CUR/110705. Manufacturing date of product was June 2018, expiry date of product was May 2021.

Fenugreek- 10% extract,batch no: **CAI/FEN/110735**. Manufacturing date of product was July 2018, expiry date of product was June 2021.

III. Standard Drug

Indomethacin- Capsule Indomethacin (Indocap) 25 mg, obtained from Jagsonpal Pharmaceuticals India. Administered at a dose of 3mg/kg.

Cyclophosphamide-TabCyclophosphamide(Cycloxan) 50 mg, obtained from Biochem Pharmaceutical industries Ltd. Lucknow, administered at a dose of 7 mg/kg.

The all drugs were given once daily orally after dissolving in normal saline with the help of a feeding cannula.

Parameters assessed:

- Body weight –By weighing balance
- Paw volume –By Vernier calipers
- Ankle diameter –By Vernier calipers
- Serum TNF-α – By ELISA kit
- Arthritic index

Research methodology

Total 60 Wistar albino rats were used in this study. Animals were acclimatized to the surroundings for 12 days, during which they were given a normal pellet diet and water ad libitum**.** Then animals were divided into 10 groups of 6 animals each and to be treated as follows:

Day 0-Baseline parameters (arthritic index, body weight, paw volume, ankle diameter, blood sampling) were assessed in all groups, normal saline was injected innormal control group (group I), while rest groups (group II to group X) 0.1 ml CFA was injectedintradermally in the footpad of their right hind paw to induce arthritis.

Day 5-All parameters reassessed in group I to group VI and treatment started in group III-VI. Primary lesion developed.

Day 12-All parameters reassessed in groups I, II, VII – X. Treatment started in groups VII – X.

Day 15-All parameters reassessed in groups I, II, III–VI. Treatment stops in group III to VI.

Day 22-All parameters reassessed in groups I, II, VII–X. Treatment stopped in groups VII–X.

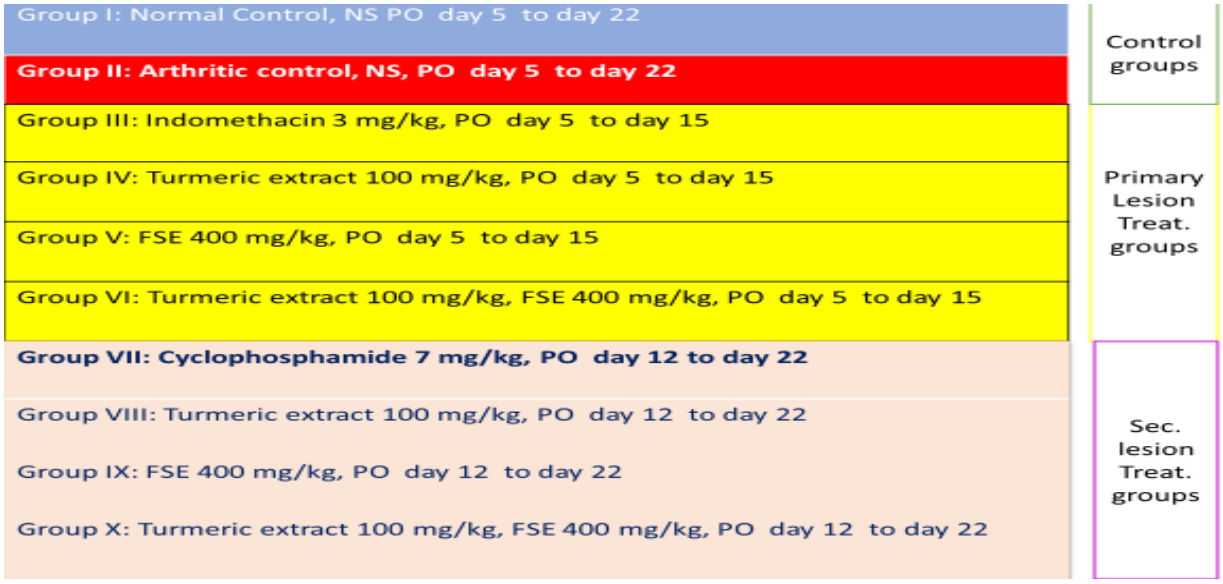

Fig 1:Treatment for different groups

Statistical analysis: All data were presented in the form of mean±SD**.** ANOVA was applied to evaluate statistically significant difference in baseline and pre-treatment values among all groups. Pre and post treatment difference were evaluated by paired T-test in same group and between groups by Tukey's post hoc test.

Observations and Result

Following observations were seen on primary lesion parameters.

Effect of drugs in primary lesion parameters from day 5 to 15

Following observations were found after statistical analysis-

Effect of drugs on body weight: Effect of drugs shown in Fig 2, table 1

Fig 2: Showing body weight on day 0, 5, 15

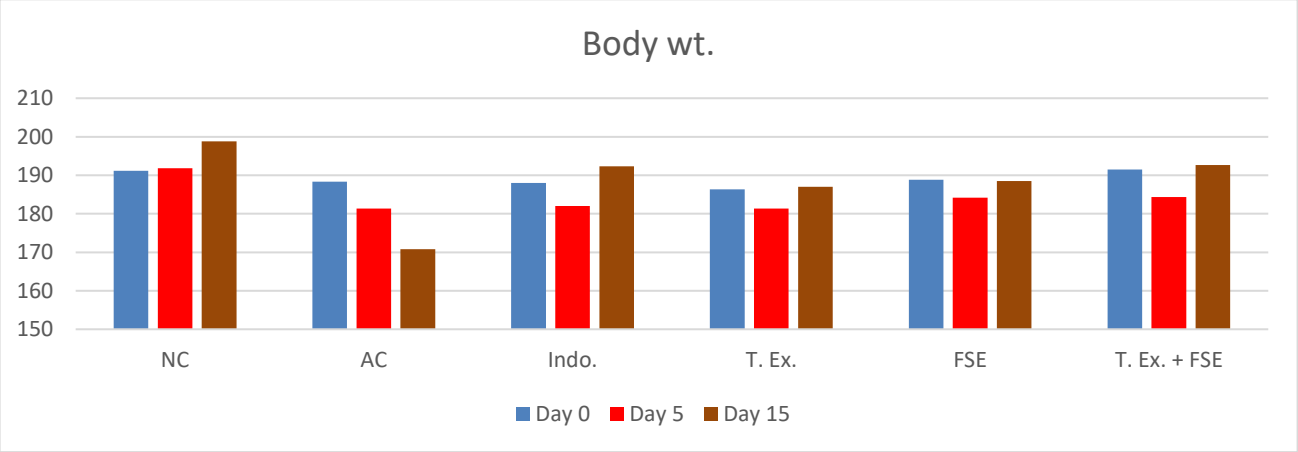

Table 1: Showing change in body weight with respect to standard drug indomethacinon day 5, 15.

Variables	Groups		Day 0- 5		Day 15-15	
			Mean Difference	P Value	Mean Difference	P Value
Body Weight	Indo. Vs	NC	-9.833	0.049	-6.000	0.347
		AC	0.667	1.000	21.500	<0.001
		T. Ex.	-0.667	1.000	5.833	0.377
		FSE	-2.167	0.984	4.667	0.615
		T. EX+FSE	-3.833	0.839	-0.333	1. 000

Effect of drugs on paw volume-Effect of drugs shown in Fig 3, table 2

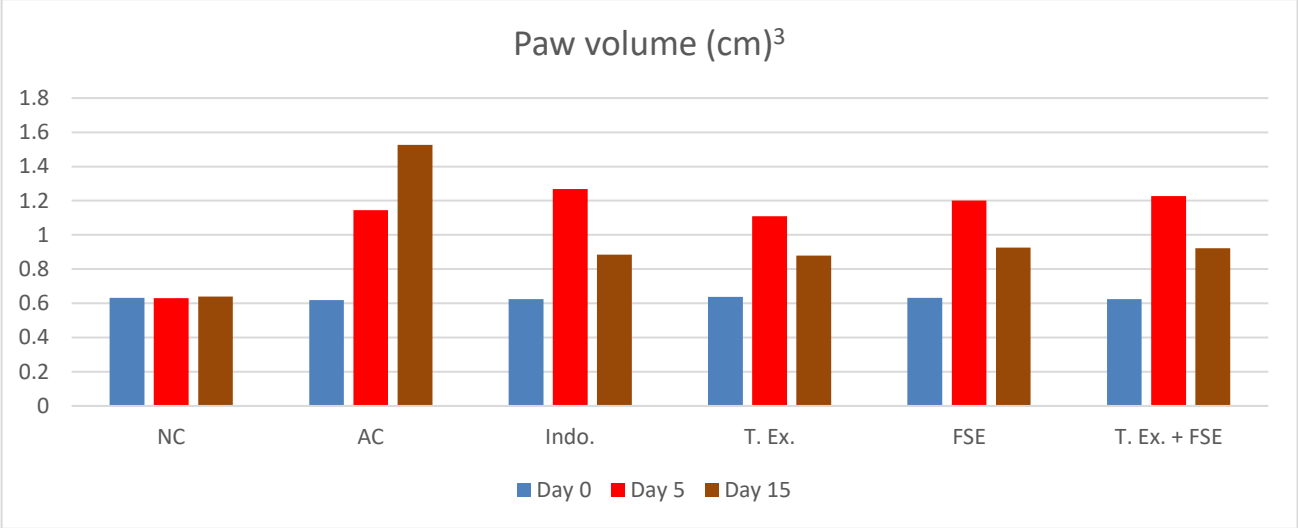

Fig 3 : Showing paw volume on day 0, 5, 15.

Table 2: Showing change in paw volume with respect to standard drug indomethacin on day 5, 15.

Variables	Groups		Day 5		Day 15	
			Mean Difference	P Value	Mean Difference	P Value
Paw volume	Indo. Vs	NC	0.632	<0.001	0.246	<0.001
		AC	0.124	0.889	-0.643	<0.001
		T. Ex.	0.160	0.742	0.005	1.000
		FSE	0.068	0.991	-0.041	0.665
		T. EX+FSE	0.041	0.999	-0.038	0.737

Effect of drugs on ankle diameter: Effect of drugs shown in Fig 4, table 3

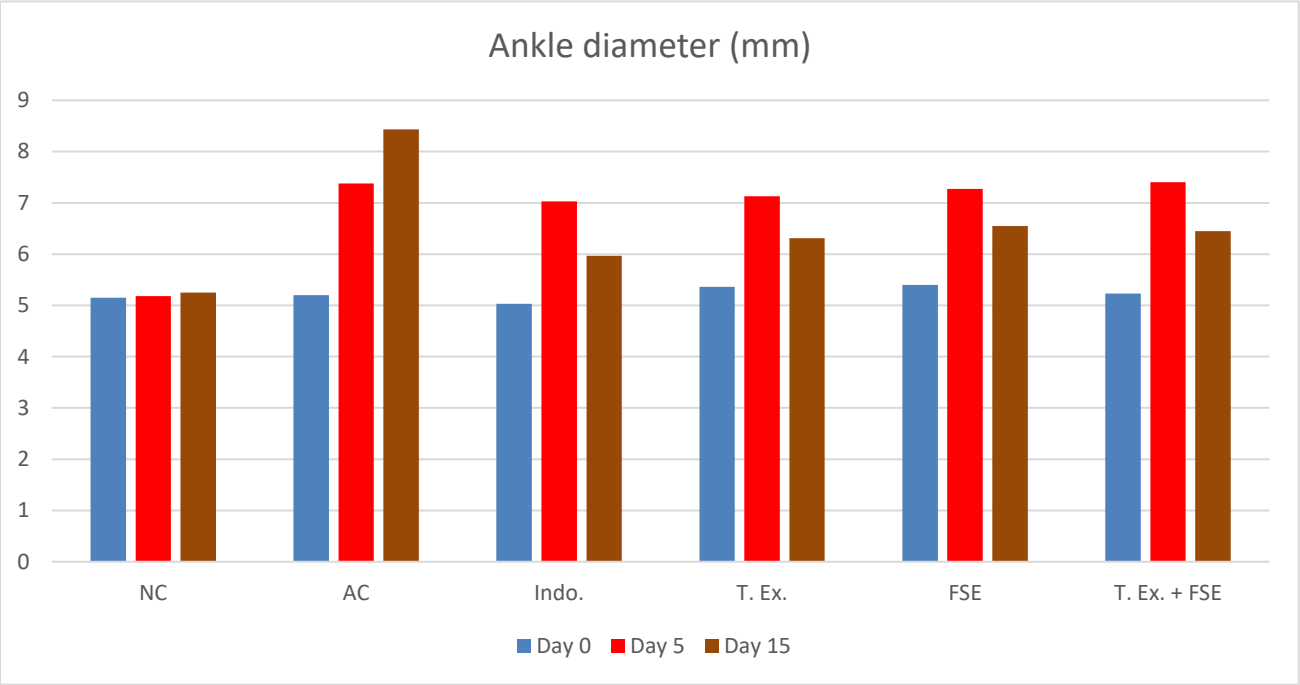

Fig 4: Ankle diameter on day 0, 5, 15

Table 3:Showing change in ankle diameter with respect to standard drug indomethacin, on day 5, 15.

Variables	Groups		Day 5		Day 15	
			Mean Difference	P Value	Mean Difference	P Value
Ankle Diameter	Indo. Vs	NC	1.850	0.005	0.717	0.229
		AC	-0.350	0.974	-2.467	<0.001
		T. Ex.	-0.100	1.000	-0.350	0.870
		FSE	-0.233	0.996	-0.583	0.442
		T. Ex.+ FSE	-0.367	0.969	-0.483	0.639

Effect of drugs on serum TNF-α: Effectof drugs shown in table 4, Fig 5

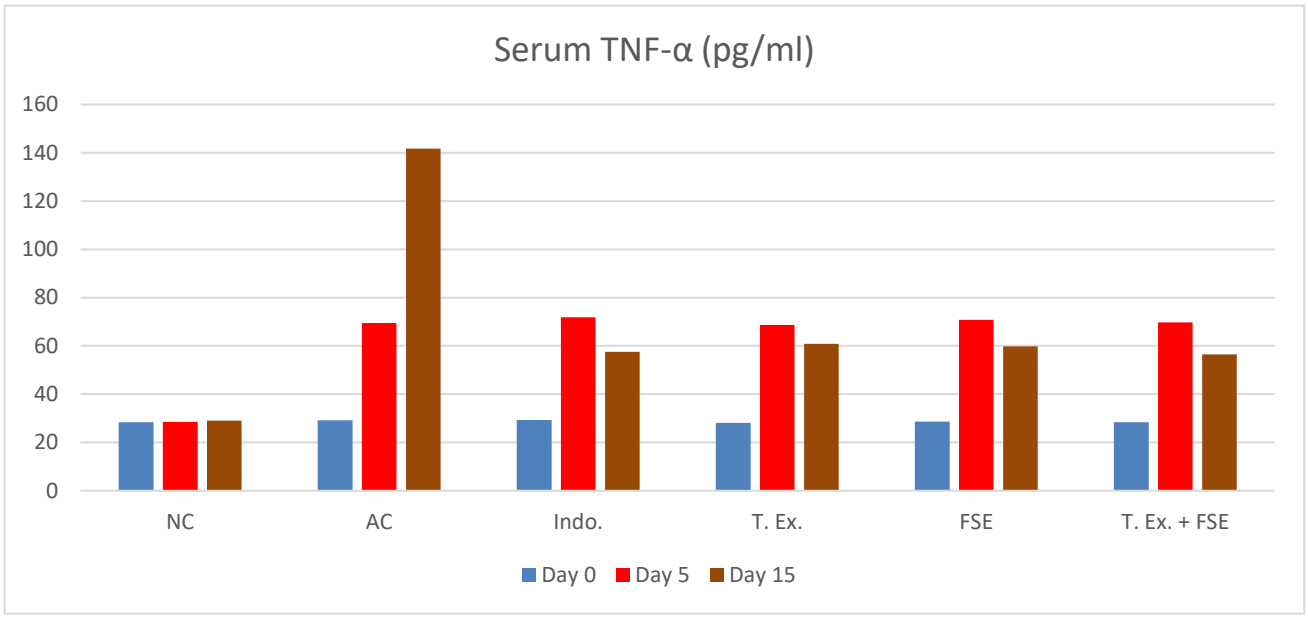

Fig 5: Serum TNF-α on day 0, 5, 15

Table 4: Showing change in serum TNF-α with respect to standard drug Indomethacin, on day 5, 15.

Variables	Groups		Day 5		Day 15	
			Mean Difference	P Value	Mean Difference	P Value
Serum TNF-α	Indo. Vs	NC	43.333	<0.001	28.500	<0.001
		AC	2.333	0.277	-84.167	<0.001
		T. Ex.	3.167	0.061	-3.333	0.921
		FSE	1.000	0.935	-2.333	0.982
		T. EX+FSE	2.167	0.354	1.000	1.000

Effect of drugs on Arthritic Index: Effect of drugs shown in table 5, Fig 6

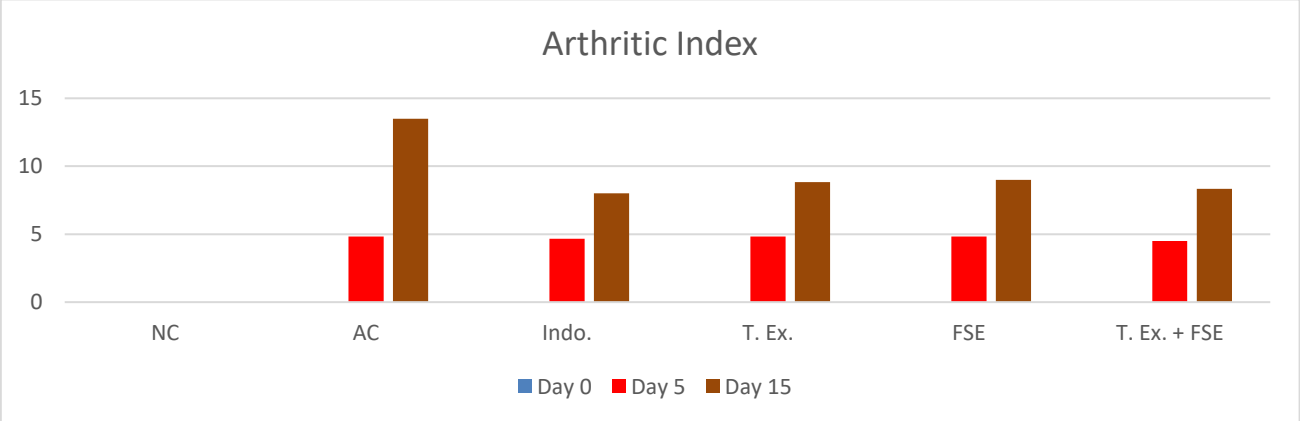

Fig 6 : Arthritic Index on day 0, 5, 15

Table 5:Showing change in arthritic index with respect to standard Indomethacin on day 5,15

Variables	Groups		Day 5		Day 15	
			Mean Difference	P Value	Mean Difference	P Value
Arthritic Index	Indo. Vs	NC	4.667	<0.001	8.000	<0.001
		AC	-0.167	0.999	-5.500	<0.001
		T. Ex.	-0.17	1.000	-0.833	0.220
		FSE	-0.167	0.999	-1.000	0.089
		T. Ex+ FSE	0.167	0.999	-0.333	0.937

Effect of drugs in secondary lesion parameters from day 12 to day 22: Following observations were found on day 12 and day 22.

Effect of drugs on body weight: Effect of drugs shown in table 6 and Fig 7

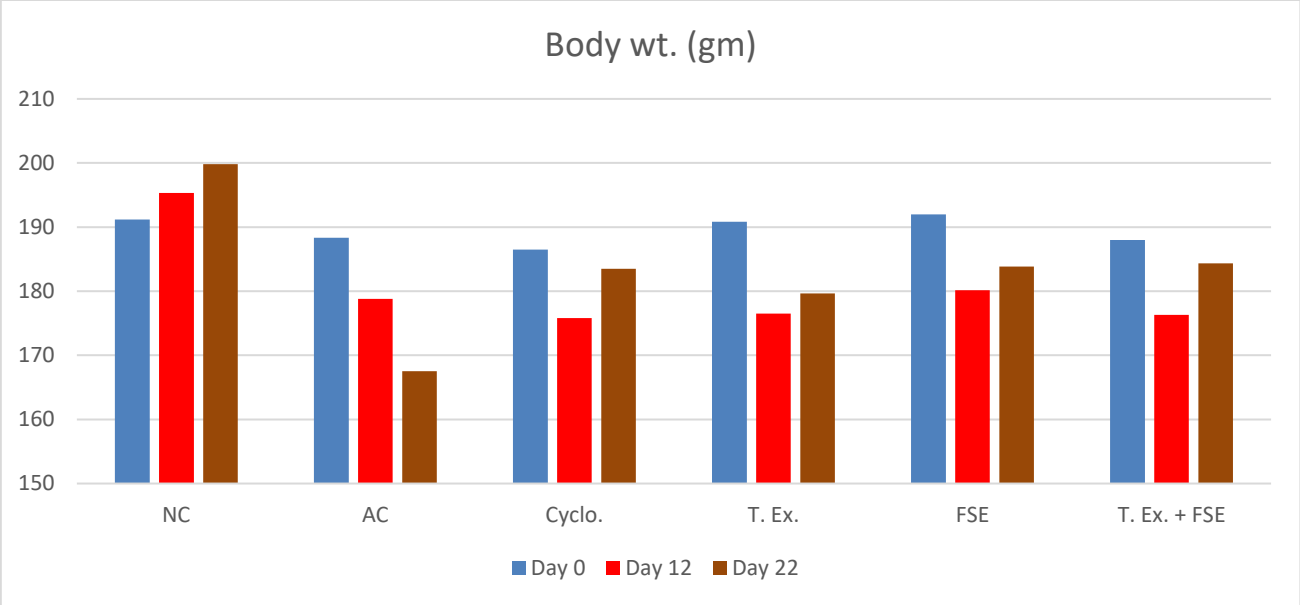

Fig 7: Body weight on day 0, day 12, day 22

Table 6: Showing change in body weight with respect to Standard drug cyclophosphamide (cyclo.), on day 12, 22.

Variables	Groups		Day 12		Day 22	
			Mean Difference	P Value	Mean Difference	P Value
Body Weight	Cyclo. Vs	NC	-19.500	<0.001	-16.333	<0.001
		AC	-3.000	0.873	16.000	<0.001
		T. Ex.	-0.667	1	3.833	0.674
		FSE	-4.333	0.60	-0.333	1
		T. EX+ FSE	-0.500	1	-0.833	0.999

Effect of drugs on paw volume: Effect of drugs shown in table 7 and Fig 8

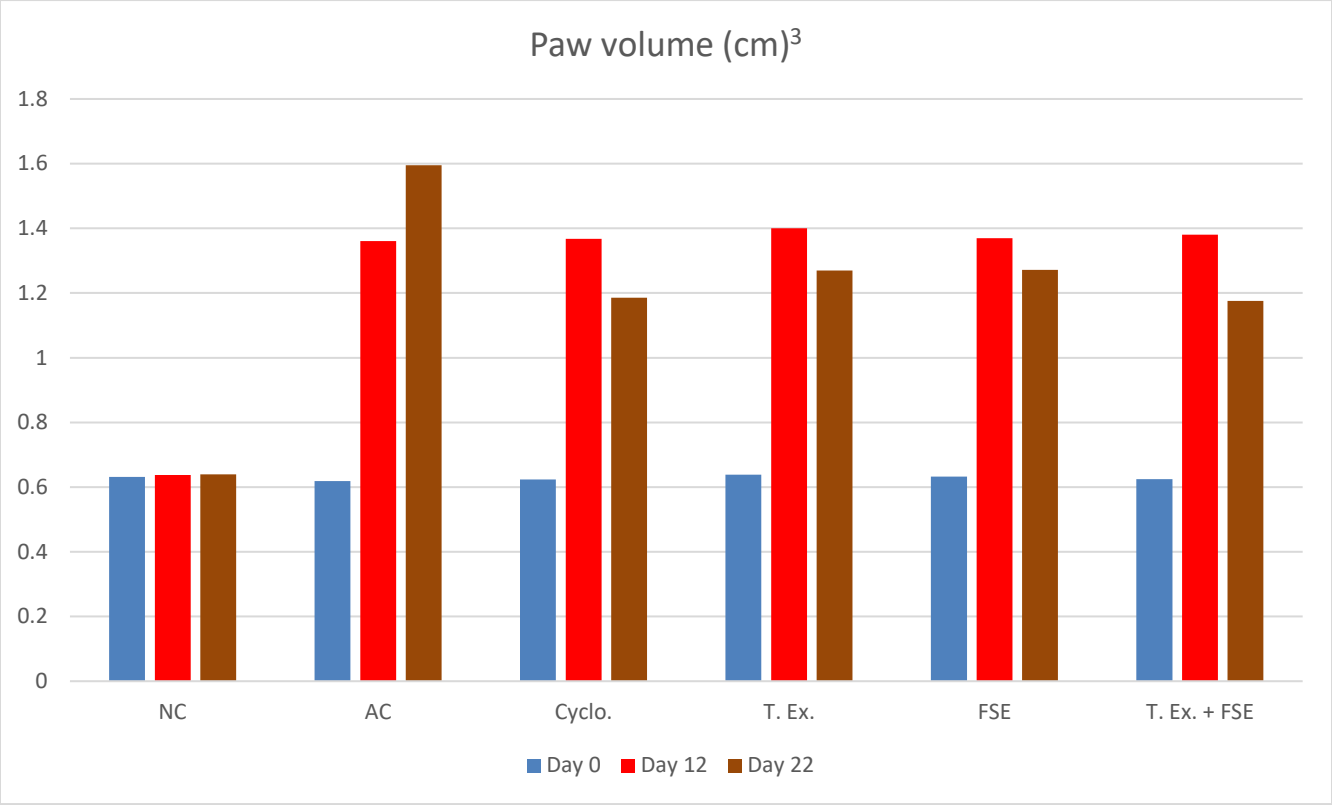

Fig 8: Paw volume on day 0, day 12, day 22

Table 7: Showing change in paw volume with respect to standard drug cyclophosphamide(cyclo), on day 12, 22.

Variables	Groups		Day 12		Day 22	
			Mean Difference	P Value	Mean Difference	P Value
Paw Volume	Cyclo. Vs	NC	0.731	<0.001	0.547	<0.001
		AC	.007	0.999	-0.409	<0.001
		T. Ex.	-0.032	0.446	-0.084	0.547
		FSE	-0.004	1.000	-0.086	0.531
		T. EX + FSE	-0.015	0.950	0.010	1.000

Effect of drugs on ankle diameter:Effect of drugs shown in table 8 and Fig 9.

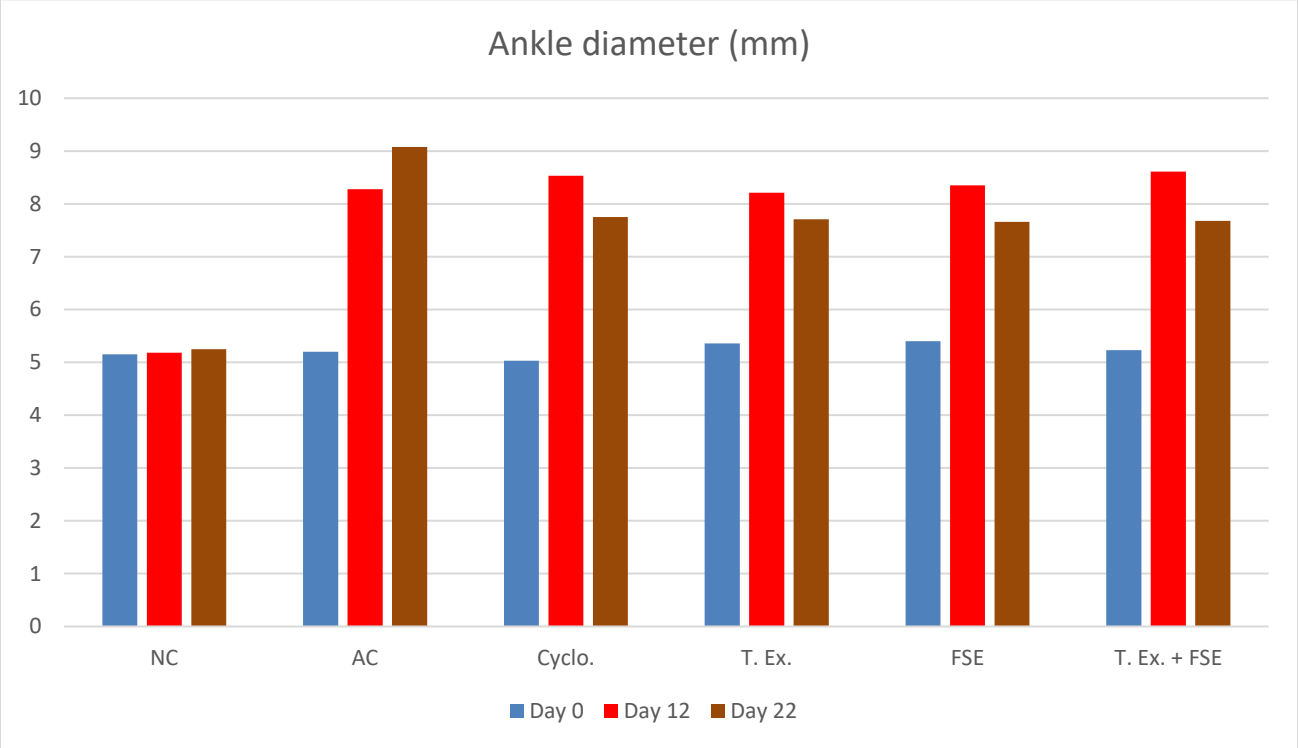

Fig 9: Ankle diameter on day 0, day 12, day 22

Table 8: Showing change in ankle diameter with respect to standard drug cyclophosphamide (cyclo), on day 12, 22.

Variables	Groups		Day 12		Day 22	
			Mean Difference	P Value	Mean Difference	P Value
Ankle Diameter	Cyclo. Vs	NC	3.350	<0.001	2.500	<0.001
		AC	0.250	0.476	-1.333	<0.001
		T. Ex.	0.317	0.230	0.033	1.000
		FSE	0.183	0.769	0.083	0.999
		T. EX+FSE	-0.083	0.990	0.067	1.000

IV) Effect of drugs on Arthritic Index

Effect of drugs shown in table 9, Fig 10

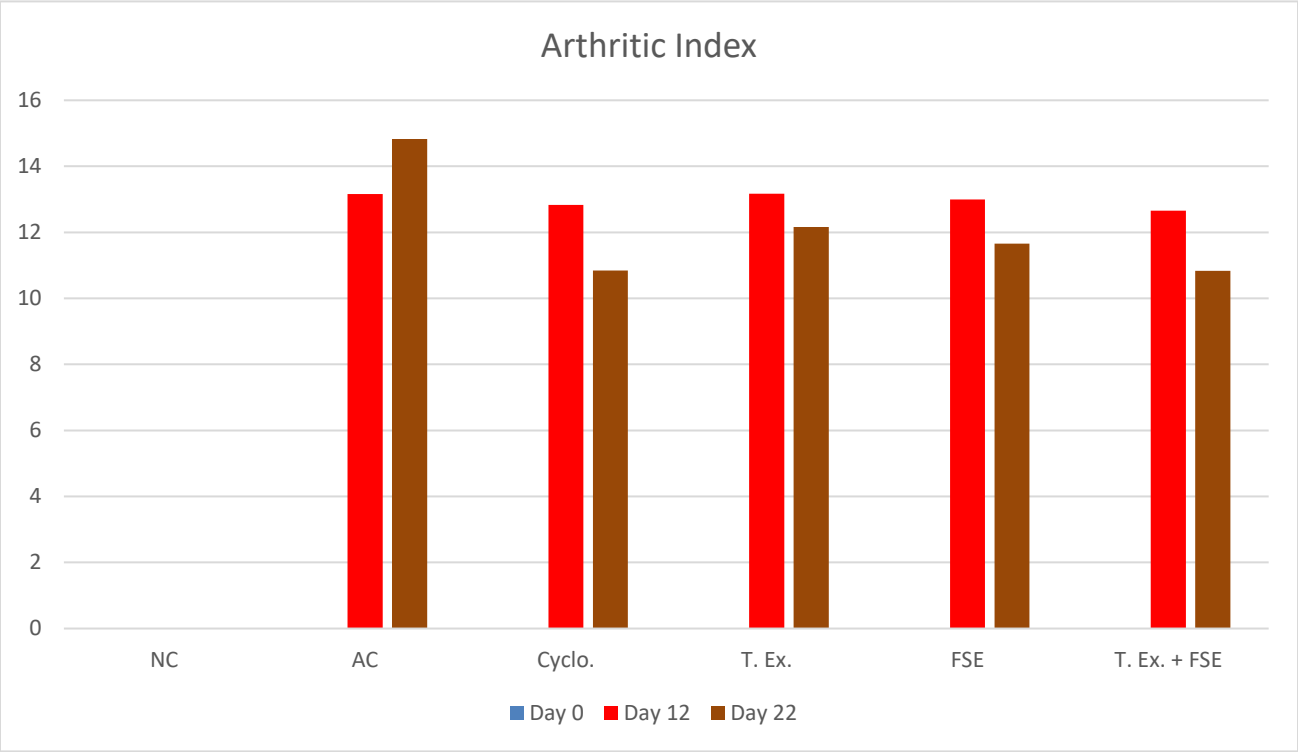

Fig 10: Arthritic index on day 0, day 12, day 22

Table 9: Showing change in arthritic index with respect to standard drug cyclophosphamide (cyclo), on day 12, 22.

Variables	Groups		Day 12		Day 22	
			Mean Difference	P Value	Mean Difference	P Value
Arthritic index	Cyclo. Vs	NC	12.833	<0.001	10.833	<0.001
		AC	-0.333	0.979	-4.000	<0.001
		T. Ex.	-0.333	0.979	-1.333	0.115
		FSE	-0.167	0.999	-0.833	0.567
		T. EX+ FSE	0.167	0.999	0	1.000

V) Effect of drugs on serum TNF-*α*:Effectof drugs shown in table 10, Fig 11

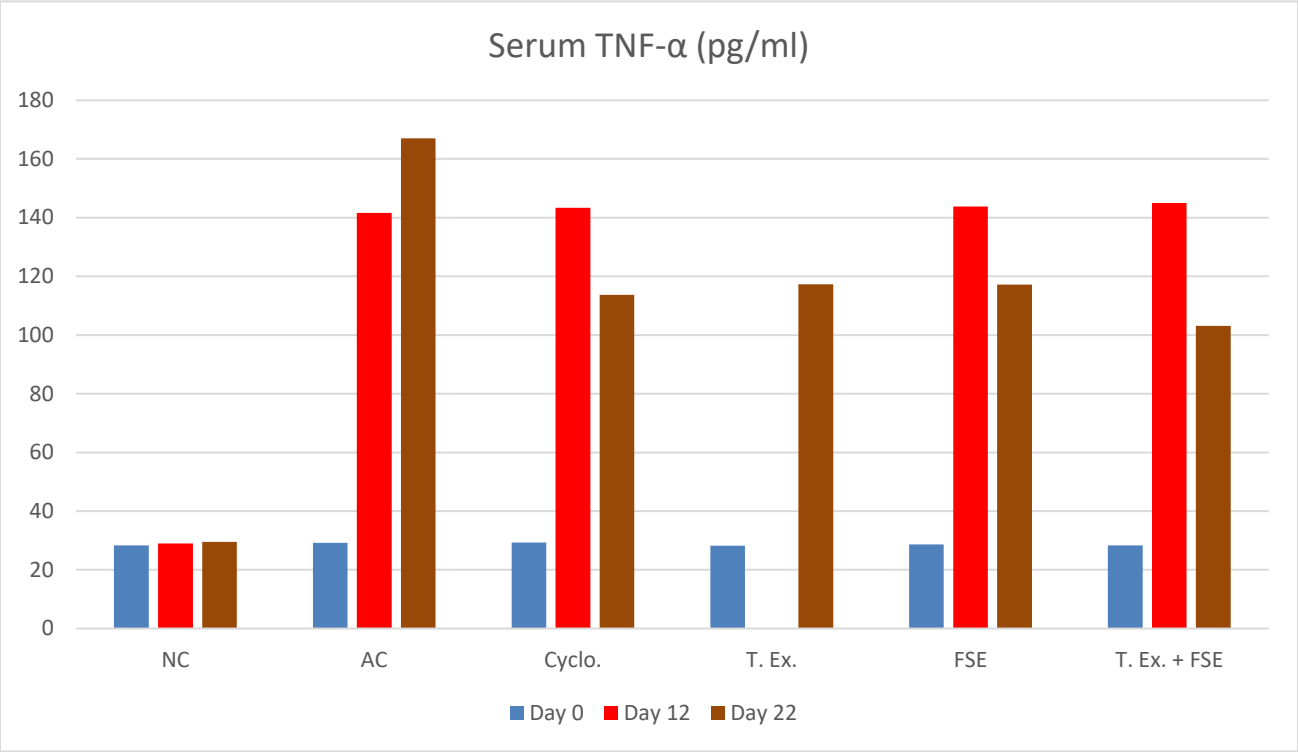

Fig 11: Serum TNF-α on day 0, day 12, day 22

Table 10: Showing change in serum TNF-α with respect to standard drug cyclophosphamide (cyclo), on day 12, 22.

Variables	Groups		Day 12		Day 22	
			Mean Difference	P Value	Mean Difference	P Value
TNF-α	Cyclo. Vs	NC	12.833	<0.001	10.833	<0.001
		AC	-0.333	0.979	-4.000	<0.001
		T. Ex.	-0.333	0.979	-1.333	0.115
		FSE	-0.167	0.999	-0.833	0.567
		T. EX+FSE	0.167	0.999	0	1.000

Discussion

In this study, we attempted to explore the anti-arthritic effect of well-known herbs Turmeric (*Curcuma longa*) and Fenugreek seeds (*Trigonellafoenumgraecum)* on CFA induced arthritis in experimental Wistar rats. Test drugs were compared with the standard drugs, Indomethacin in primary lesion groups and Cyclophosphamide in secondary lesion groups.With the results of this study it becomes clear that Turmeric extract and fenugreek seed extract have anti-arthritic potential. Marked suppression of paw volume, ankle diameter, serum TNF-α, arthritic index, increase in body weight was observed after treatment with Turmeric extract and Fenugreek seed extract alone and in combination.

On comparison with standard drug indomethacin, cyclophosphamide,statistically significant difference was not observed among all treatment groups. So, we can say that both test drugs alone and in combination

are comparable to standard drugs. Turmeric extract decreases serum TNF-α level. This observation is in accordance with a previous study [12-17]. The decreased serum TNF-α statistically significant in fenugreek seed extract treated rats. This observation is in accordance with a previous study. This suggests that the fenugreek seed extract decrease serum TNF-α level. This may explain the serum TNF-α of group-II rats [18,19,20]. This observation is in accordance with a previous study by Suresh P, Kavitha CN et.al**.** These effects may be seen due to:anti-inflammatory, ability to reduce TNF-α level, as elevated TNF-α promotes, influx of leukocytes at the site of inflammation, activation of synovial fibroblasts, angiogenesis. The decreased in paw volume, paw thickness and ankle diameter of turmeric extract treated rats may be due to its anti-inflammatory, down-regulating the activity of cyclooxygenase-2 (COX--2), lipoxygenase, and inducible nitric oxide synthase enzymes, inhibits the production of the inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin (IL) -1, -2, -6, -8, and -12[16, 21]. This may explain the increased paw volume of group-II rats. These findings are in accordance with the previous studies [22-25]

Conclusion

On the basis of findings in the present study, the following conclusions may be drawn regarding the potential effectiveness of Turmeric extract and Fenugreek seed extract on CFA induced arthritic rats-

- Anti-arthritic effect of turmeric extract at dose 100 mg/kg and fenugreek seed extract 400 mg/kg was clearly evident in primary lesionparameters, it is comparable to standard drug indomethacin and secondary lesion parameters it is comparable to standard drug cyclophosphamide.
- In the light of above evidences, the Turmeric extract 100 mg/kg and Fenugreek seed extract 400 mg/kg alone and in combination might have a potential usefulness as an alternative to conventional therapy in further management of rheumatoid arthritis.

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