ORIGINAL RESEARCH

Effects of Piper longum in complete freund's adjuvant induced rheumatoid arthritis and its interaction with cyclosporine-A in rats

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ABSTRACT

Introduction: Piper longum has been studied and found to have potentially beneficial anti-arthritic properties. Consequently, we intended to investigate the effects of an aqueous extract of the fruit of Piper longum on complete Freund's adjuvant-induced arthritic changes in Wistar rats, as well as its interaction with cyclosporine A. **Methodology:** A total of 48 Wistar rats were divided into 8 groups each containing 6 rats: Group I (control), Group II (Rheumatoid Arthritis control (complete Freund's adjuvant [CFA])), Group III (CFA+ Piper longum 200mg/kg/day), Group IV (CFA+ Piper longum 400mg/kg/day), Group V (CFA+ Cyclosporine A 10mg/kg/day (subtherapeutic dose)), Group VI (CFA+ Piper longum 200mg/kg/day + cyclosporine A 10mg/kg/day), Group VII (CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day) and Group VIII (CFA+ Cyclosporine A 15mg/kg/day (Standarddrug)). They received a normal pellet diet and water ad libitum. They were allowed to get acclimatized to the new environment for 1 week and assessed for 28 days. Arthritis parameters were assessed on the 14th and 28th days. **Results:** Except for group 1, weight, Proinflammatory cytokine (TNF-), Anti-inflammatory cytokine (IL-10), Nuclear Factor Kappa Beta (NF-), Malondialdehyde (MDA), and Superoxide dismutase (SOD) varied significantly among groups. The arthritis index decreased in all treatment groups. When changes in left and right paw volume were measured, all treatment groups exhibited a significant reduction in left and right paw volume. Similarly, the diameter of the ankle decreased in the treatment groups. **Conclusion:** In conclusion, the aqueous extract of Piper longum possesses potentially useful anti-arthritic activity.

Keywords: Anti-rheumatoid activity, Piper longum, Complete Freund's adjuvant, Wistar Rats, Proinflammatory cytokine, Anti-inflammatory cytokine.

INTRODUCTION

Rheumatoid Arthritis (RA) is a systemic autoimmune disorder with an unknown aetiology that is characterised by protracted inflammation and immune cell infiltration of the synovium. Symptoms of rheumatoid arthritis include musculoskeletal distress, joint swelling, and joint stiffness. Patients with RA are at a greater risk than the general population for infection, respiratory disease, osteoporosis, cardiovascular disease, cancer, and death[1]. Chronic, progressive rheumatoid arthritis is associated with substantial morbidity and premature mortality. It occurs between 0.3% and 1% of the time worldwide and is more prevalent in affluent nations. The estimated adult prevalence in India is 0.75 %[2]. The therapeutic goals include minimising synovial inflammation, alleviating pain, preventing joint injury, and preventing a decline in physical function. Several proinflammatory cytokines, including tumour necrosis factor (TNF), interleukin-6 (IL-6), and high-sensitivity C-reactive protein (hs CRP), are positively correlated with the pathogenesis of rheumatoid arthritis (RA), whereas IL-10 is negatively correlated. Biological disease-modifying antirheumatic drugs (bDMARDs) are large proteins that target extracellular inflammatory mediators specifically. These include pro-inflammatory cytokines and membrane-associated immunological proteins[3]. No existing treatment is capable of completely resolving rheumatoid arthritis, and the available medications

have severe side effects. Therefore, there is an imperative need for the development and evaluation of novel chemicals for the treatment of rheumatoid arthritis. Piper longum or Pipali, which was primarily used as a spice and condiment, is now a component of medicine, according to several studies. This plant has immunomodulatory, anti-inflammatory, antioxidant, analgesic, and anticancer properties[4]. The active ingredient in Piper longum is piperine, which enhances the bioavailability of a variety of drugs, including sulfadiazine, tetracycline, streptomycin and many more. As piperine has a significant impact on increasing the bioavailability of medications, it can be combined with therapeutic agents to reduce the effective dose of pharmaceuticals, thereby reducing their side effects[5]. This study was planned to investigate the effects of an aqueous extract of the fruit of Piper longum on complete freund's adjuvant-induced arthritic changes and its interaction with cyclosporine A in Wistar rats.

MATERIAL AND METHODS

The study was conducted in the Department of Pharmacology and Therapeutics, King George's Medical University, Lucknow, for twelve months. After getting approval from the institutional animal ethics committee (IAEC). Adult healthy Wistar rats weighing 180-250 gm were used in the study. 48 rats were obtained from CPSCEA–certified animal houses [IITR, Lucknow]. They were allowed to access food and water ad libitum and were kept in the institutional animal house under a temperature-controlled environment [25°± 2° Celsius] with a 12-hour light-dark cycle. They were allowed to get acclimatized to the new environment for 1 week. Rats were randomly divided into 8 groups, each containing 6 rats and assessed for 28 days.Each group's animals were sacrificed under a high dose of sodium pentobarbital (100 mg/kg i.p.). On the 14^{th} and 28^{th} days, rheumatoid arthritis parameters were evaluated. Each limb was rated on a scale of 0 to 4, with a maximum total score of 16. The following were the criteria for evaluation: Arthritis Index- No oedema or visual changes (score=0), Slight oedema and limited erythema (score=1), Light oedema and erythema (score=2), Obvious oedema and substantial erythema (score=3), and Severe oedema and extensive erythema (score=4). Using a digital plethysmometer available in the experimental laboratory of the department of pharmacology and therapeutics, paw volume changes were measured. The diameter of the ankle was measured using a vernier calliper just above the lateral malleolus. As rheumatoid arthritis has an impact on body weight, it was assessed every three days using a digital weighing scale. Using the orbital plexus capillary method, blood samples were drawn from all rodents on day 14 as a baseline reading, and on day 28 for serum TNF-, IL-10, MDA, SOD, and NF-k levels. ELISA was utilised to measure cytokines. Malondialdehyde (MDA) and Superoxide dismutase (SOD) were determined using the thiobarbituric acid (TBA) and Marklund and Marklund method using pyrogallol, respectively.

STATISTICAL ANALYSIS

Data were entered in Microsoft Excel and analyzed using statistical software SPSS version 26 (SPSS Inc., Chicago, IL, USA). The continuous variables were evaluated by mean (standard deviation) or range value when required. The dichotomous variables were presented in number/frequency and were analyzed using Chi-square. A p-value of < 0.05 or 0.001 was regarded as significant.

RESULTS

In the group CFA+ Piper longum 400 mg/kg/day + cyclosporine A 10mg/kg/day and CFA+ Cyclosporine A 15 mg/kg/day, the standard drug showedthe most significantweight reduction[Table-1]. Similar were the results for the level of cytokines and biochemical markers[Table2 and 3]. Further, the table-4 showed that all the treatment groups had a decrease in the arthritis index, while it was only significant in the groups: CFA+ Piper longum 400 mg/kg/day + cyclosporine A 10 mg/kg/day, ($p<0.0001$*), followed by CFA+ Cyclosporine A 15 mg/kg/day, Standard drug ($p<0.0001$*) and CFA+ Piper longum 200 mg/kg/day + cyclosporine A 10 mg/kg/day ($p=0.0398$*). When the left paw volume change was assessed onthe 28^{th} day, all the treatment groups showed a significant decrease in left paw volume change, where groups: CFA+ Cyclosporine A 15 mg/kg/day, Standard drug ($p<0.0001$*), followed by CFA+ Piper longum 400 mg/kg/day + Cyclosporine A 10 mg/kg/day ($p<0.0001$*) and CFA+ Piper longum 200 mg/kg/day + Cyclosporine A 10 mg/kg/day ($p<0.0001$*) were the most significant. Group CFA+ Piper longum 400 mg/kg/day + cyclosporine A 10 mg/kg/day had the most significant volume change in the right paw. The mean ankle diameter was comparable among controls. However, all the treatment groups showed a significant decrease in ankle diameter. The group CFA+ Cyclosporine A 15mg/kg/day, Standard drug, followed by CFA+ Piper longum 200mg/kg/day + cyclosporine A 10mg/kg/day and CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day were the most significant groups ($p<0.0001$*) [Figure-1-4].

Table-1: Effect of Piper longum on weight in complete freund's adjuvant-induced arthritis in rats.

WEIGHT (g)					
	AT DAYS 14		AT DAYS 28		P-VALUE
	MEAN±SD	MEDIAN [QUARTILE]	MEAN±SD	MEDIAN [QUARTILE]	
Control	163.2±8.37	167.5 [153.3-169.3]	183.3±7.89	186.5 [173.8-189.0]	t=4.28 **p=0.0016***
Rheumatoid Arthritis control	161.8±7.11	160.5 [156.0-171.0]	188.3±8.24	191.5 [178.5-195.3]	t=5.96 **p=0001***
CFA+ Piper longum 200mg/kg/day	162.5±7.56	163.0 [155.5-170.0]	186.8±9.26	187.0 [178.8-195.5]	t=4.98 **p=0.0005***
CFA+ Piper longum 400mg/kg/day	162.8±5.31	163.0 [157.5-167.8]	170.0±5.29	170.5 [164.5-175.3]	t=2.35 **p=0.0404***
CFA+ Cyclosporine A 10mg/kg/day	161.5±7.17	163.0 [154.0-168.3]	145.7±8.36	147.5 [138.8-152.5]	t=3.51 **p=0.0055***
CFA+ Piper longum 200mg/kg/day + cyclosporine A 10mg/kg/day	160.8±7.57	162.5 [153.0-167.5]	168.2±9.28	172.5 [157.3-174.8]	t=2.28 **p=0.0455***
CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day	161.2±7.22	160.0 [154.5-168.3]	180.2±7.46	182.5 [172.8-186.3]	t=4.48 **p=0011***
CFA+ Cyclosporine A 15mg/kg/day, Standard drug	160.5±7.29	160.0 [154.3-166.5]	128.2±10.65	130.5 [122.3-135.8]	t=6.13 **p=0.0001***

Table-2: Effect of Piper longum on immunomodulatory cytokinesin complete freund's adjuvant-induced arthritis in rats.

	PROINFLAMMATORY CYTOKINE TNF-α (pg/ml)			ANTI-INFLAMMATORY CYTOKINE – IL-10 (pg/ml)		
	At 14 days	At 28 days	p-value	At 14 days	At 28 days	p-value
Control	48± 6.69	44.63± 5.53	t=0.9510 p=0.3640	287.27± 19.96	279.2± 5.98	t=0.9487 p=0.3651
Rheumatoid Arthritis control	150.5± 10.25	176.52±14.58	t=3.576 **p=0.0050***	52.17± 6.91	31.17± 2.91	t=6.861 **p<0.0001***
CFA+ Piper longum 200mg/kg/day	147.2± 9.2	122.57±11.53	t=4.090 **p=0.0022***	55.52± 7.87	93± 22.3	t=3.882 **p=0.0030***
CFA+ Piper longum 400mg/kg/day	148.7± 7.5	96.53± 6.59	t=12.80 **p<0.0001***	53.62± 9.63	109± 21.76	t=5.701 **p=0.0001***
CFA+ Cyclosporine A 10mg/kg/day	151.5± 9.64	75.52± 7.52	t=15.22 **p<0.0001***	54.87± 8.53	177.8± 18.59	t=14.02 **p<0.0001***
CFA+ Piper longum 200mg/kg/day + cyclosporine A 10mg/kg/day	149.83±9.38	68.83± 5.38	t=18.35 **p<0.0001***	52.63± 4.52	196.8± 26.9	t=12.95 **p<0.0001***
CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day	148.17±7.28	48.17± 7.28	t=23.79 **p<0.0001***	54.29± 5.52	260.2± 10.64	t=42.08 **p<0.0001***
CFA+ Cyclosporine A 15mg/kg/day, Standard drug	147.5± 8.21	53.42± 5.21	t=23.70 **p<0.0001***	55.36± 4.63	242.5± 10.15	t=41.09 **p<0.0001***

Table-3: Effect of Piper longum on biochemical markers in complete freund's adjuvant-induced arthritis in rats.

	MALONDIALDEHYDE (MDA) nmol/mL			SUPEROXIDE DISMUTASE (SOD) Units/mg		
	At 14 days	At 28 days	p-value	At 14 days	At 28 days	p-value
Control	0.87± 0.21	0.85± 0.17	t=0.1813 p=0.8597	10.12± 1.07	10.63± 1.26	t=0.7557 p=0.4672
Rheumatoid Arthritis control	2.65± 0.81	3.09± 0.89	t=0.8956 p=0.3915	3.68± 0.98	2.88± 0.23	t=1.947 p=0.0802
CFA+ Piper longum 200mg/kg/day	2.83± 0.98	2.05± 0.61	t=1.655 p=0.1289	3.88± 1.13	6.55± 1.14	t=4.074 **p=0.0022***
CFA+ Piper longum 400mg/kg/day	2.87± 0.93	1.47± 0.78	t=2.825 **p=0.0180***	3.55± 1.14	5.07± 1.05	t=2.402 **p=0.0372***
CFA+ Cyclosporine A 10mg/kg/day	2.76± 0.95	1.67± 0.44	t=2.550 **p=0.0288***	4.03± 1.05	7.65± 1.3	t=5.306 **p=0.0003***
CFA+ Piper longum 200mg/kg/day + cyclosporine A 10mg/kg/day	2.89± 1.01	1.27± 0.42	t=3.628 **p=0.0046***	3.67± 1.3	9.78± 2.59	t=5.164 **p=0.0004***
CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day	3.03± 1.03	0.99± 0.21	t=4.754 **p=0.0008***	4.16± 1.59	10.03± 2.15	t=5.655 **p=0.0002***
CFA+ Cyclosporine A 15mg/kg/day, Standard drug	2.98± 0.96	1.05± 0.26	t=4.753 **p=0.0008***	4.03± 1.15	9.88± 2.23	t=5.711 **p=0.0002***

Table-4: Effect of Piper longum on arthritis parameters in complete freund's adjuvant-induced arthritis in rats.

	ARTHRITIS INDEX			LEFT HIND PAW VOLUME CHANGES (mL)		
	At 14 days	At 28 days	p-value	At 14 days	At 28 days	p-value
Control	0.33± 0.52	0.33± 0.52	t=0.000 p>0.9999	0.23± 0.04	0.22± 0.03	t=0.4899 p=0.6348
Rheumatoid Arthritis control	6.33± 1.21	11.17± 0.98	t=7.614 **p=0.0001***	1.31± 0.05	1.48± 0.12	t=3.203 **p=0.0094***
CFA+ Piper longum 200mg/kg/day	6.67± 1.37	6.17± 0.75	t=0.4511 p=0.7842	1.33± 0.08	1.07± 0.06	t=6.369 **p<0.0001***
CFA+ Piper longum 400mg/kg/day	6.83± 1.75	6.33± 0.52	t=0.6709 p=0.5175	1.29± 0.09	0.89± 0.09	t=7.698 **p<0.0001***
CFA+ Cyclosporine A 10mg/kg/day	6.2± 1.98	6.17± 0.75	t=0.6709 p=0.5175	1.31± 0.11	0.92± 0.09	t=6.721 **p<0.0001***
CFA+ Piper longum 200mg/kg/day + cyclosporine A 10mg/kg/day	6.19± 1.17	4.83± 0.75	t=2.362 **p=0.0398***	1.28± 0.11	0.64± 0.04	t=13.39 **p<0.0001***
CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day	5.5± 1.25	0.67± 0.52	t=8.739 **p<0.0001***	1.31± 0.08	0.28± 0.08	t=22.3 **p<0.0001***
CFA+ Cyclosporine A 15mg/kg/day, Standard drug	5.67± 1.52	0.83± 0.41	t=7.531 **p<0.0001***	1.32± 0.08	0.32± 0.06	t=24.74 **p<0.0001***
	RIGHT HIND PAW VOLUME CHANGES (mL)			Ankle Diameter (mm)		
Control	0.14±	0.15±	t=0.6794	6.43±	6.62±	t=0.8315

	0.02	0.03	p=0.5123	0.37	0.42	p=0.4251
Rheumatoid Arthritis control	0.81± 0.06	0.92± 0.06	t=3.175 **p=0.0099***	13.83± 1.49	13.97± 0.61	t=0.2130 p=0.8356
CFA+ Piper longum 200mg/kg/day	0.78± 0.05	0.71± 0.05	t=1.993 **p=0.0742***	13.67± 1.36	11.3± 0.92	t=3.536 **p=0.0054***
CFA+ Piper longum 400mg/kg/day	0.79± 0.06	0.5± 0.06	t=7.705 **p<0.0001***	12.98± 1.23	9.42± 0.77	t=6.009 **p=0.0001***
CFA+ Cyclosporine A 10mg/kg/day	0.81± 0.05	0.54± 0.05	t=8.468 **p<0.0001***	13.56± 1.36	9.33± 1.2	t=5.713 **p=0.0002***
CFA+ Piper longum 200mg/kg/day + cyclosporine A 10mg/kg/day	0.82± 0.04	0.18± 0.04	t=27.71 **p<0.0001***	13.69± 1.48	7.35± 0.85	t=9.099 **p<0.0001***
CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day	0.8± 0.05	0.16± 0.05	t=29.11 **p<0.0001***	12.49± 1.29	6.94± 0.84	t=8.831 **p<0.0001***
CFA+ Cyclosporine A 15mg/kg/day, Standard drug	0.81± 0.06	0.21± 0.06	t=21.91 **p<0.0001***	13.38± 1.45	7.25± 0.65	t=9.449 **p<0.0001***

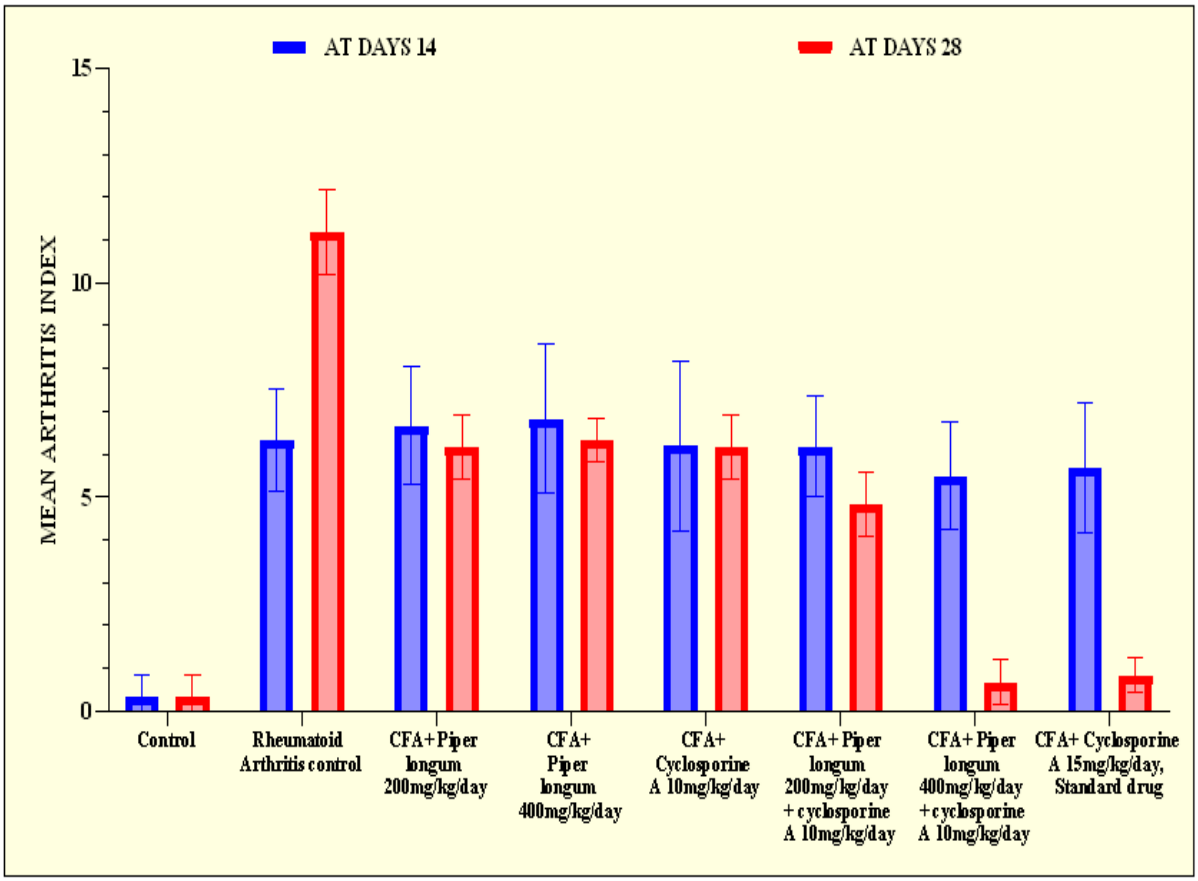

Figure-1: Effect of Piper longum on arthritis index in complete freund's adjuvant-induced arthritis in rats.

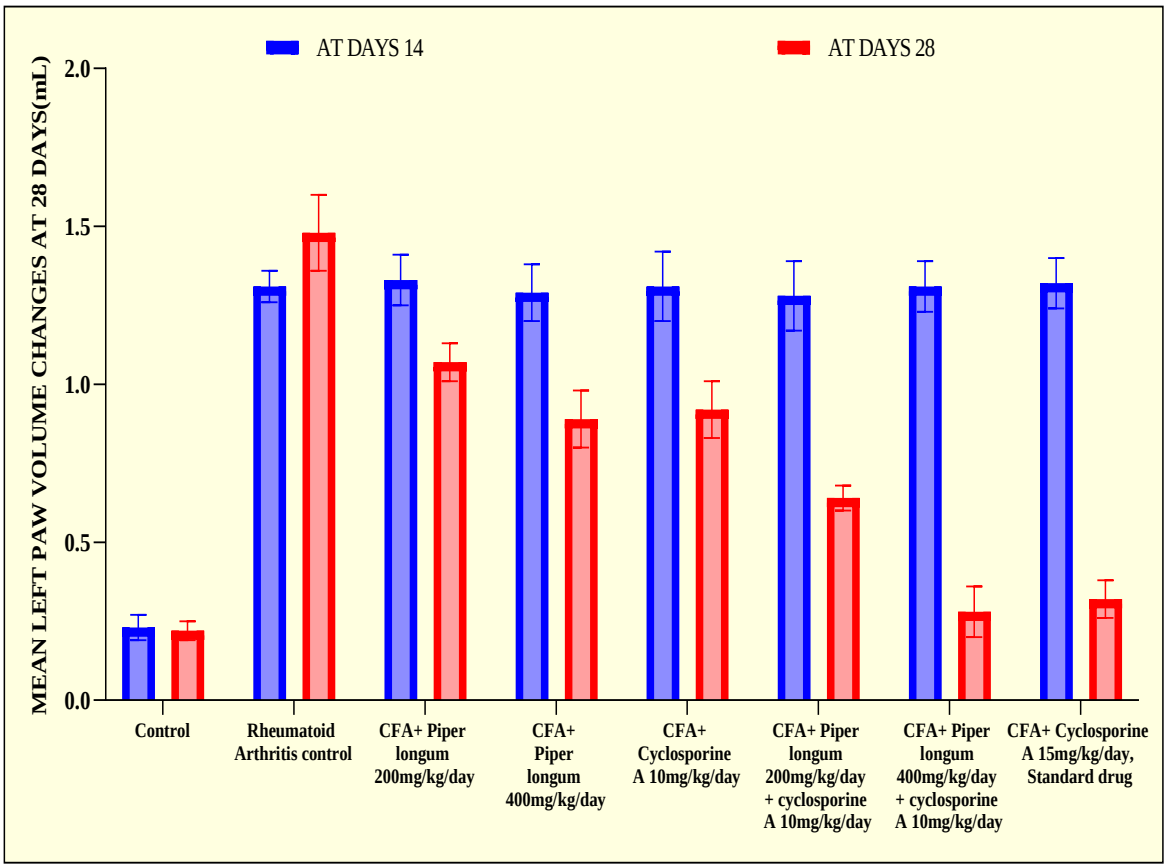

Figure-2: Effect of Piper longum on change in volume of left paw at 28 days in complete freund's adjuvant-induced arthritis in rats.

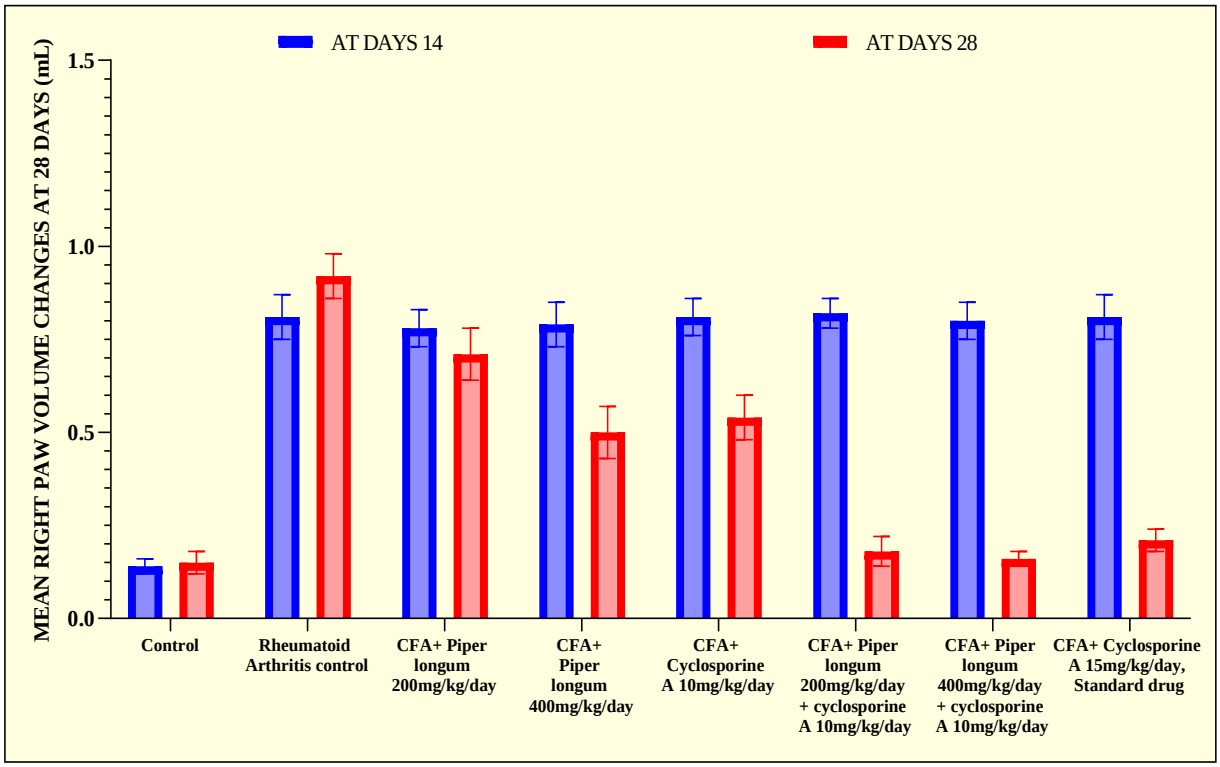

Figure-3: Effect of Piper longum on change in volume of right hind paw at 28 days in complete freund's adjuvant-induced arthritis in rats.

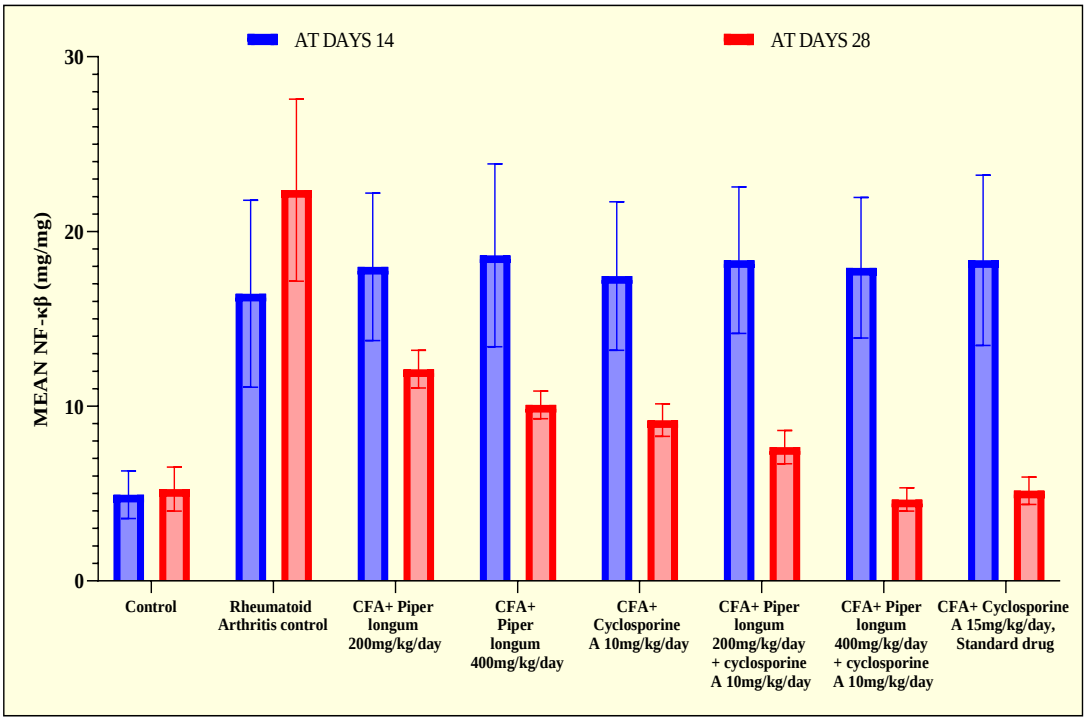

Figure-4: Graphical representation of the effect of Piper longum on Level of Nuclear Factor Kappa Beta (NF-κβ) in complete freund's adjuvant induced arthritis in rats.

Welling of soft tissue adjacent or around the joint is associated with arthritis and reduction of this swelling is an expected parameter or action of drugs used in arthritis. The swelling of soft tissue was fond to be more in the control animals group as compared to the Diclofenac sodium

DISCUSSION

In the present study, CFA administration showed a significantly increased body weight in rats compared with the control group. Treatment of Piper longum 200mg/kg/day after CFA administration showed a significant decrease in body weight as compared to CFA administered group. While the other groups also showed a decrease in body weight compared to the CFA-treated group. A maximum decrease in weight was found in CFA+ Cyclosporine A 15mg/kg/day group. All the data of each group was found to be significant as compared to the baseline. **Yende SR et al.** showed normal body weight when treated with piperine[6].The present study revealed that CFA increased the level of MDA. All other groups showed a significant decrease in the level of MDA as compared to CFA treated group. Treatment of rats with CFA+ Piper longum 400mg/kg/day and CFA + cyclosporine A 10mg/kg/day + Piper longum 400mg/kg/day showed a greater decrease in the level of MDA as compared to those treated with CFA+ Piper longum 200mg/kg/day and CFA+ Piper longum 200mg/kg/day + cyclosporine A 10mg/kg/day. Treatment of rats with CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day showed the best result in decreasing the level of MDA as compared to other groups which were nearly equal to the control group. **Umar Set al.,** induced arthritis in male Wistar rats by collagen-induced arthritis (CIA) method. Piperine was administered at a dose of 100 mg kg-1 and indomethacin at 1 mg kg-1 body weight once daily for 21 days[7]. Results showed that Piperine was effective in bringing significant changes in the level of MDA. In the current study, CFA administration has shown a significantly reduced activity of endogenous antioxidants (SOD) compared with the control group. Treatment with CFA+ Piper longum 200mg/kg/day increased the SOD as compared to CFA treated group. Treatment of rats with CFA+ Piper longum 400mg/kg/day showed a greater increase in SOD as compared to 200mg/kg/day of Piper longum. CFA+ Cyclosporine A 10mg/kg/day treatment significantly increases the SOD but is lesser than Piper longum 400mg/kg/day and Piper longum 200mg/kg/day. Treatment of rats with CFA+ Piper longum 200mg/kg/day + cyclosporine A 10mg/kg/day and CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day shows much better results than Piper longum treatment alone. Treatment of rats with standard drug (Cyclosporine A 15mg/kg/day) increases the SOD levels which are approximately equal to CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day treatment. According to **Hussein SA et al.,** superoxide dismutase is considered the first line of defence against the deleterious effects of oxygen radicals in cells, where it scavenges ROS by catalyzing the dismutation of superoxide to H_2O_2 and O_2[8].According to **Barua CC et al.,** thedecreased

levels of the antioxidants might be due to the excessive consumption to defend against oxidative damage and/ or inactivation or inhibition of the enzymes by hydrogen peroxide[9]. Treatment with PDL and PHF significantly blunted the depletion of GSH, SOD and CAT, probably by competing for scavenging of free radicals and preserving the integrity of cellular membranes.Our result demonstrated that treatment of rats with CFA increased the level of TNF- α and NF-κβ as compared to the control group. Treatment of rats with CFA+ Piper longum 200mg/kg/day decreased the TNF- α and NF-κβ levels as compared to CFA treated group. Treatment of rats with CFA+ Piper longum 400mg/kg/day decreased the TNF- α and NF-κβ in a better way as compared to 200mg/kg/day of Piper longum alone. CFA+ Cyclosporine A 10mg/kg/day treatment significantly decreased the TNF- α and NF-κβ but more than Piper longum 400mg/kg/day and Piper longum 200mg/kg/day. Treatment of rats with CFA+ Piper longum 200mg/kg/day + cyclosporine A 10mg/kg/day and CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day showed a greater decrease than the Piper longum treatment alone. Treatment of rats with standard drug (Cyclosporine A 15mg/kg/day) decreases the TNF- α, which is approximately equal to CFA+ Piper longum 200mg/kg/day + cyclosporine A 10mg/kg/day treatment. Group VII showed better results than all the groups. The comparison of Group I with other groups showed statistical significance. Our result is much similar to that of **Tong, Z et al.,** which revealed that TNF-α in the serum of Freud's adjuvant-induced arthritis (AA) model rats was high[10]. According to **Garg AK et al.,**TNF- α - induced free radical generation like H_2O_2 activates inflammatory signalling pathway, including NF-κβ in vascular cells and regulates the expression of cell adhesion molecules on endothelial cells and hence play an important role in various inflammatory diseases[11].**Rahman A et al.,** reported that chloroform extracts of P. longum inhibited the TNF-α induced expression of intercellular adhesion molecule-1 (ICAM-1)[12].Furthermore, the extract inhibited the adherence of neutrophils to endothelial monolayer by inhibiting the TNF- α - induced expression of ICAM-1, Vascular cell adhesion molecule-1 (VCAM-1) and E-selectin in a dose- and time-dependent manner. Also, chloroform extracts of P. longum significantly inhibited the TNF- α -induced activation of NF-κβ. **Yende SR et al.,** and **Barua CC et al.,** study demonstrated that PHF has the potential to suppress the various aspects of inflammatory immune responses and molecular events in adjuvant-induced arthritis by modulating antioxidants and pro-inflammatory cytokines and attenuating the expression of pro-inflammatory mediators and transcription factor (NF-κβ) in the synovial joint[6,9].Our result also showed a significant decrease in the level of IL-10 in the CFA-administered group as compared to the control group. All the other groups showed a significant increase in the level of IL-10 as compared to CFA treated group. Treatment of rats with CFA+ Piper longum 200mg/kg/day + cyclosporine A 10mg/kg/day and CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day showed much better results than the Piper longum treatment alone. Treatment of rats with standard drug (Cyclosporine A 15mg/kg/day) increased the IL-10, but it is in a lesser amount than CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day. Thus, the result overall revealed that Group VII showed much better results than other treatments. This study is in support of the previous result of **UmarS et al.,**piperine shifts the balance of cytokines toward a bone-protecting pattern that acts to both lower levels of TNF-a, IL-1b, and raises the levels of IL-10[7].**Kumar S et al.,** showedthe anti-inflammatory effect of piperine by inhibiting proinflammatory cytokines and through inhibition of the adhesion of neutrophils to endothelial monolayer[13]. Hence, it is plausible to suggest that part of the beneficial anti-inflammatory and cartilage/bone protective effects of piperine may be mediated through the inhibition of proinflammatory cytokines. Our study revealed that CFA increased the Arthritis Index as compared to the control group. Treatment of rats with CFA+ Piper longum 200mg/kg/day and CFA+ Piper longum 400mg/kg/day also showed reduced Arthritis Index. Treatment of rats with the standard drug cyclosporine A 15mg/kg/day reduced the Arthritis Index as compared to the CFA group. Treatment with CFA+ Piper longum 200mg/kg/day + cyclosporine A 10mg/kg/day, CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day), and CFA+ Cyclosporine A 15mg/kg/day (Standard drug) also showed the better result as compared to CFA treated group but overall CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day showed the better result. Our study is similar to the **Mahdi HJ et al.**study, which showed that moringa extract showed less inflammatory and arthritis symptoms with a statistically significant decrease in arthritic index[14].The result of the present study is that CFA treatment increased the volume of the right hind paw as compared to the control group. While all the others showed a reduction in the volume of the right hind paw as compared to CFA treated group. Treatment with CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day showed that the volume of the right hind paw is approximately equal to the control group. Similarly, **Tong Z et al.,**showed that Arthritic scores and the change of right-back paw swelling perimeter are indexes for evaluating the anti-arthritic activity of many medicines[10]. These indexes were used for estimating the effect of CMC at different doses in this research. **Yende SR et al.,** showed that an aqueous extract of P. longum significantly suppressed the swelling of the paws[6]. Reduction of paw swelling in the P. longum-treated

rats from the third week onward may be due to immunological protection rendered by the plant extract. **Barua CC et al.,** showed that arthritis was induced by sub planter injections of FCA into the footpad of the left hind paw[9]. The increase in the paw volumes in both FCA-injected and non-FCA-injected paws indicates the primary and secondary arthritic lesions respectively. Administration of PDL and PHF significantly suppressed the paw swelling induced by the FCA.In the current study, CFA administration showed a significant increase in ankle diameter compared with the control group. Treatment with CFA+ Piper longum 200mg/kg/day decreased the ankle diameter as compared to CFA treated group. Treatment of rats with CFA+ Piper longum 400mg/kg/day decreased ankle diameter in a much better way as compared to 200mg/kg/day of Piper longum. CFA+ Cyclosporine A 10mg/kg/day treatment significantly decreased ankle diameter but was lesser than Piper longum 400mg/kg/day and Piper longum 200mg/kg/day. Treatment of rats with CFA+ Piper longum 200mg/kg/day + cyclosporine A 10mg/kg/day and CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day shows much better results than the Piper longum treatment alone. Treatment of rats with CFA+ Piper longum 400mg/kg/day + cyclosporine A 10mg/kg/day showed a major reduction in the ankle diameter as compared to the CFA group, which was approximately near to the control group. In contrast to our result**Noh AS et al.,** the CFA 5.0 and CFA 7.5 groups showed a statistically significant increase in spontaneous activities and the development of thermal hyperalgesia but no change in body weight or food intake, no onset of tactile allodynia or changes in haematological indices, and no statistically significant morphological changes in joint histology[15].We can say that treatment of Piper longum as an adjuvant with cyclosporine A could provide a better result as compared to the use of cyclosporine A alone. However, we may recommend the use of Piper longum in arthritis patients after some more elaborative research.

CONCLUSION

The aqueous extract of Piper longum possesses potentially useful anti-arthritic activity. The results encourage the view that Piper longum can be useful alone or as an add-on therapy in the treatment of rheumatoid arthritis but more specific and longer duration experimental and clinical studies are required to further substantiate the findings of the present study.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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None

CONSENT

As per international standards or university standards written participant consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standards or university standards written ethical permission has been collected and preserved by the author(s).

ABBREVIATIONS

CsA-Cyclosporine
APl - Piper Longum
MRI- Magnetic Resonance Imaging
CFA-Complete Freund's Adjuvant
CDC-Center for Disease Control and Prevention
CRP- C-Reactive Protein

REFERENCES

1. Sparks, JA. Rheumatoid Arthritis. Ann Intern Med. 2019 Jan 1;170(1):ITC1-ITC16.https://doi.org/10.7326/AITC201901010
2. Pal R, Chaudhary MJ, Tiwari PC, Babu S, Pant KK. Protective role of theophylline and their interaction with nitric oxide (NO) in adjuvant-induced rheumatoid arthritis in rats. International Immunopharmacology. 2015 Dec 1;29(2):854-62.https://doi.org/10.1016/j.intimp.2015.08.031
3. Law ST, Taylor PC. Role of biological agents in treatment of rheumatoid arthritis. Pharmacological research. 2019 Dec 1;150:104497. https://doi.org/10.1016/j.phrs.2019.104497
4. Gani HM, Hoq MO, Tamanna T. Ethnomedicinal, phytochemical and pharmacological properties of Piper longum (Linn). Asian Journal of Medical and Biological Research. 2019 Apr 22;5(1):1-7. https://doi.org/10.3329/ajmbr.v5i1.41038
5. Guo Z, Xu J, Xia J, Wu Z, Lei J, Yu J. Anti-inflammatory and antitumour activity of various extracts and compounds from the fruits of Piper longum L. Journal of Pharmacy and Pharmacology. 2019 Jul;71(7):1162-71.https://doi.org/10.1111/jphp.13099
6. Yende SR, Sannapuri VD, Vyawahare NS, Harle UN. Antirheumatoid activity of aqueous extract of Piper longum on freunds adjuvant-induced arthritis in rats. Int J Pharm Sci Res. 2010;1(9):129-33.https://www.semanticscholar.org/paper/Antirheumatoid-activity-of-aqueous-extract-of-Piper-Yende-Sannapuri/e6ab107defcf02bdbdc074d3ccfa19336d60c340
7. Umar S, Sarwar AH, Umar K, Ahmad N, Sajad M, Ahmad S, Katiyar CK, Khan HA. Piperine ameliorates oxidative stress, inflammation and histological outcome in collagen induced arthritis. Cellular Immunology. 2013 Jul 1;284(1-2):51-9.https://doi.org/10.1016/j.cellimm.2013.07.004
8. Hussein SA, Ragab OA, El-Eshmawy MA. Renoprotective effect of dietary fish oil on cyclosporine A: Induced nephrotoxicity in rats. Asian Journal of Biochemistry. 2014;9(2):71-85.https://doi.org/10.3923/ajb.2014.71.85
9. Barua CC, Bodduluru LN, Haloi P, Purukayastha A, Patowary P, Hussain M, Bora M. Anti-arthritic and anti-inflammatory activity of a polyherbal formulation

against Freund's complete adjuvant induced arthritis in Wistar rats. Indian J. of Traditional Knowledge. 2017 Jul 1;16(3):482-9. https://www.semanticscholar.org/paper/Anti-arthritic-and-anti-inflammatory-activity-of-a-Barua-Bodduluru/949c83f1902ed932cb44d91c7dadf572fda38153

10. Tong Z, Cheng L, Song J, Wang M, Yuan J, Li X, et al. Therapeutic effects of Caesalpinia minax Hance on complete Freund's adjuvant (CFA)-induced arthritis and the anti-inflammatory activity of cassane diterpenes as main active components. Journal of Ethnopharmacology. 2018 Nov 15;226:90-6.https://doi.org/10.1016/j.jep.2018.08.011
11. Garg AK, Aggarwal BB. Reactive oxygen intermediates in TNF signaling. Molecular immunology. 2002 Dec 1;39(9):509-17.https://doi.org/10.1016/S0161-5890(02)00207-9
12. Rahman A, Al-Reza SM, Sattar MA, Kang SC. Potential roles of essential oil and extracts of Piper chaba Hunter to inhibit Listeria monocytogenes. Records of Natural Products. 2011;5(3):228-37.https://acgpubs.org/doc/2018080616363929_RNP-1008-289.pdf
13. Kumar S, Singhal V, Roshan R, Sharma A, Rembhotkar GW, Ghosh B. Piperine inhibits TNF-α induced adhesion of neutrophils to endothelial monolayer through suppression of NF-κB and IκB kinase activation. European journal of pharmacology. 2007 Dec 1;575(1-3):177-86.https://doi.org/10.1016/j.ejphar.2007.07.056
14. Mahdi HJ, Khan NA, Asmawi MZ, Mahmud R, Vikneswaran A, Murugaiyah L. In vivo anti-arthritic and anti-nociceptive effects of ethanol extract of Moringa oleifera leaves on complete Freund's adjuvant (CFA)-induced arthritis in rats. Integrative medicine research. 2018 Mar 1;7(1):85-94.https://doi.org/10.1016/j.imr.2017.11.002
15. Noh AS, Chuan TD, Khir NA, Zin AA, Ghazali AK, Long I, Ab Aziz CB, Ismail CA. Effects of different doses of complete Freund's adjuvant on nociceptive behaviour and inflammatory parameters in polyarthritic rat model mimicking rheumatoid arthritis. Plos one. 2021 Dec 8;16(12):e0260423. https://doi.org/10.1371/journal.pone.0260423