

Anti-Arthritic and Anti Inflammatory Activity of Beta Caryophyllene against Freund's Complete Adjuvant Induced Arthritis in Wistar Rats

Vijayalaxmi A¹,
Vasudha Bakshi²,
Nazia Begum¹, Kowmudi V¹,
Naveen Kumar Y¹,
Yogesh Reddy¹

Abstract

Aim of the study: Beta-Caryophyllene is a potent phyto cannabinoid which is reported to have anti-inflammatory activity. In view of its potent anti-inflammatory activity, the present study was designed to evaluate its anti-arthritic activity.

Materials and strategies: The Beta-Caryophyllene was tested against Complete Freund's adjuvant (CFA) induced arthritic rats. Arthritis assessment, paw volume were measured. And radiological examination was performed. On day 22, the animals were sacrificed; Hind paw joint was extracted for histopathology and biochemical parameters like anti-oxidants, total protein, lipid peroxidation, serum nitrates, SGOT, SGPT, ALP, and Bilirubin were examined.

Results: Beta-Caryophyllene significantly decreased the arthritis which was evident with arthritis index, paw volume as well as the maintenance of biochemical parameters. The histopathology and radiology also revealed the control in inflammation with Beta-Caryophyllene.

Conclusion: The present study is suggestive that Beta-Caryophyllene has prominent anti-arthritic activity which may be attributed to its anti-inflammatory activity.

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Introduction

Rheumatoid arthritis (RA) is a chronic, relapsing inflammatory and autoimmune multisystem illness that affects the joints, characterized by inflammation of the synovial membrane, pain and restricted joint movement [1]. Rheumatoid arthritis is systemic inflammatory disease in which the destruction of articular cartilage leads to bone deformity and loss of joint function and ultimately severe pain. It is the most common inflammatory arthritis affecting approximately 1-2% of the general population worldwide i.e. 20 million people worldwide. Incidence increases with age, with women being affected three times more than men (Arthritis Research Campaign). The risk of incidence appears to be greatest for women between 40 and 50 years of age, and for men somewhat later, which makes the rheumatoid arthritis to be an age-related immune disorder [2,3]. Rheumatoid arthritis is a chronic disease, and although rarely, a spontaneous remission may occur, the natural course is almost invariably one of persistent

symptoms, waxing and waning in intensity, and a progressive deterioration of joint structures leading to deformations and disability. While there are many treatments like non-steroidal anti-inflammatory drugs (NSAIDs) and disease modifying anti-rheumatoid arthritic drugs (DMARDs) available for Rheumatoid arthritis, up to 30% of patients fail to respond to treatment [3]. However, besides their high cost, prolonged use of many of these drugs is associated with severe adverse reactions such as gastric and duodenal ulcers, complications in the small intestine and colon can occur, which cause colitis, bleeding, perforation, stricture, and chronic problems such as iron deficiency anemia and protein loss and toxicity. A report also states that, NSAIDs treatment enhances joint destruction in arthritis and inhibits glycosaminoglycan synthesis. Recently, there has been an increasing interest in natural food for scavenging the free radicals because of their wide acceptance [4].

Some constituents of plants, particularly terpenoids, have

- 1 Dept. of Pharmacology, School of Pharmacy, Anurag Group of Institutions, Ghatkesar, Hyderabad, Telangana, India
- 2 Principal and Head of the Department, Dept. of Pharmacology, School of Pharmacy, Anurag Group of Institutions, Ghatkesar, Hyderabad, Telangana, India

Corresponding author: Vijayalaxmi A

✉ vijjianumasa07@gmail.com

Dept. of Pharmacology, School of Pharmacy, Anurag Group of Institutions, Ghatkesar, Hyderabad, Telangana, India

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been reported to be useful in the management of inflammatory diseases [5]. Among the terpenoids, β -Caryophyllene (trans-4,11,11-trimethyl-8-methylenebicyclo[7,2,0] undec-4-ene) is a sesquiterpene present in very large amounts in natural products e.g. clove oil, cinnamon leaves, and copaiba balsam, all of which have been used as natural remedies and also as fragrances (Figure.1). This compound is also known to be anti-microbial [6], anti-oxidant [6,7], and anti-carcinogenic [8] and to possess skin penetration-enhancing properties [9]. Essential oils that have Beta-Caryophyllene as a major component (30.6%) showed marked anti-inflammatory activity against carrageenan and prostaglandin E1-induced edema in rats as well as anti-arthritic activity [9,10].

The aim of the present study, therefore, was to evaluate the ameliorative effect of oral administration of Beta-Caryophyllene (Figure.1) in rats on experimental Arthritis induced by Complete Freund's adjuvant (CFA).

Materials and Methods

Chemicals

Freund's complete adjuvant (Sigma Aldrich, USA), Beta Caryophyllene, (Gift sample from Shree Bankey Bihari Lal Board Mills, Ghaziabad, Gujarat). All other chemicals and reagents used for study were of analytical grade procured from approved organization.

Animals

Male Wistar rats of body weight 200–300 g were used for the study. The animals were maintained under standard environmental conditions and were fed with standard pellet diet and water ad libitum. All the experimental procedures were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) guidelines. All the experimental procedures were approved by IAEC. (I/IAEC/LCP/011/2014/WR-30♂)

Induction of arthritis and treatment protocol

The animals were divided into six groups of six animals each as follows:

1. Group I–Vehicle Control, 2.5% w/v Tween 20, p.o; (non-arthritic);
2. Group II–Arthritic control, (0.1ml Complete Freund's adjuvant)
3. Group III– Arthritic animals treated with Beta-Caryophyllene 100 mg/kg, p.o;

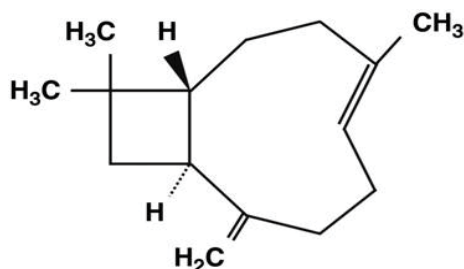


Figure 1 Structure of β -Caryophyllene.

4. Group IV– Arthritic animals treated with Beta-Caryophyllene 300 mg/kg, p.o;
5. Group V– Arthritic animals treated with standard, 10 mg/kg Aceclofenac, p.o;

Arthritic control group was given a single injection of 0.1ml Complete Freund's adjuvant in to subplantar region of right hind paw on day 1 under light ether anesthesia [12]. The dosing of all the groups started from day 1-12 once daily orally and the study was continued till day 21. Dose selection was based on previously published studies showing that oral doses of Beta-Caryophyllene used ranged from 1mg/kg to 600 mg/kg, Anti-arthritic activity of Beta-Caryophyllene was evaluated on paw volume, animal body weight and arthritic score on day 0, 3, 6, 9, 12, 15, 18 and day 21. The animals were sacrificed on day 22 to study the joint histology.

Arthritic score

The morphological feature of the arthritis like redness, swelling and erythema [12,13] was monitored by set visual criteria as follows: normal paw = 0, mild swelling and erythema of digits = 1, swelling and erythema of the digits = 2, severe swelling and erythema = 3, gross deformity and inability to use the limb = 4 on respective days. Thus, the maximum possible score for both hind paws was 8.

Paw volume

The left hind paw volumes of all animals were measured just before Freund's complete adjuvant injection on day 0 and thereafter at different time intervals till day 22 using a plethysmometer (VJ instruments) [14,15]. The change in paw volume was measured as the difference between the final and initial paw volumes.

Biochemical analysis

On day 22, blood was withdrawn by retro-orbital puncture and serum was used for estimation of Anti-oxidants like SOD, Catalase, reduced glutathione, lipid peroxidation, serum nitrates, SGOT, SGPT, Bilirubin and alkaline phosphatase [16,17].

X-ray radiography

Rats were anaesthetized by intraperitoneal injection of 50mg kg⁻¹ pentobarbitone sodium on day 22. Radiographs were taken with X-ray apparatus (PHILIPS Diagnose X-ray) operated at a voltage of 55 kV against 3.2 mA s⁻¹ with a tube-to-film distance of 110 cm for lateral projection. The severity of the joint and bone deformation was blindly scored according to the extent of osteoporosis, joint spaces, osteophytes and joint structure [18,19] on a scale of 0–4 (0 – uninjected control group with no degenerative joint changes, 1 – slight soft tissue volume, joint space, subchondral erosion, periostitis, osteolysis, subluxation, and degenerative joint changes, 2 – low to moderate soft tissue volume, joint space, subchondral erosion, periostitis, osteolysis, subluxation, and degenerative joint changes, 3 – pronounced soft tissue volume, joint space, subchondral erosion, periostitis, osteolysis, subluxation, and degenerative joint changes, 4 – excess soft tissue volume, joint space, subchondral erosion, periostitis, osteolysis, subluxation, and degenerative joint changes).

Histological analysis

The animals were sacrificed on day 22 by cervical dislocation. Ankle joints were separated from the hind paw, weighed and immersed in 10% buffered formalin for 24 h followed by decalcification in 5% formic acid, processed for paraffin embedding sectioned at 5_ thickness. The sections were stained with haematoxylin and eosin [20] and evaluated under light microscope for the presence of hyperplasia of synovium, pannus formation and destruction of joint space.

Statistical analysis

The data was analyzed by one way ANOVA followed by turkey test. The values of $P < 0.05$ were considered statistically significant [21].

Results

In the present study, rats were selected to induce arthritis because rats develop a chronic swelling in multiple joints with influence on inflammatory cells, erosion of joint cartilage and bone destruction. Within 24 h of administering the Freund's complete adjuvant cardinal signs of inflammation, hyperalgesia, swelling, and hyperaemia were evident in all animals. Since measurement of vital signs of inflammation in both hind limbs is more practical and accurate than a visual grading system [22] and also allows the effects of drug-treated groups to be expressed quantitatively, we measured the hypersensitivity, and paw edema of each rat joint.

Effect of Beta-Caryophyllene (BCP) on arthritic score

All the groups of animals administered with Freund's complete adjuvant started showing signs of clinical inflammation in one or more hind paws, which was a biphasic response (Table 1-7). The first manifestation of disease was erythema of one or more ankle joints followed by involvement of the metatarsal and interphalangeal joints. There was an initial development in the manifestations of

Table 1 Effect of Beta-Caryophyllene (BCP) on Arthritic index.

Group	Arthritic index
Group I (Vehicle)	0.00± 0.00
Group II (Complete Freund's adjuvant)	3.16± 0.30***
Group III (BCP 100mg/kg+ CFA)	1.66± 0.33###
Group IV (BCP 300mg/kg+CFA)	1.50± 0.34\$\$\$
Group V (Aceclofenac10mg/kg+CFA)	1.33± 0.42&&&

Values are expressed as mean ± SEM of n=6 animals. *** $P < 0.001$ indicates comparison of negative control group with control group. ### $P < 0.05$ indicates comparison of low dose group with negative control. \$\$\$ $P < 0.001$, indicates comparison of high dose group with negative control. &&& $P < 0.001$, && $P < 0.01$ indicates comparison of standard with negative control.

Table 2 Effect of Beta-Caryophyllene on Paw volume.

Groups/ Days	Paw volume (mL)							
	1st	3rd	6 th	9 th	12th	15th	18 th	21st
Control	1.070 ± 0.079	1.43± 0.10	1.42± 0.04	1.44± 0.09	1.44± 0.11	1.557± 0.09	1.240± 0.11	1.26± 0.05
CFA	1.250± 0.03***	3.48± 0.15***	2.93± 0.091***	2.69± 0.14***	2.42± 0.26***	2.33± 0.14***	2.25± 0.160***	2.18± 0.05***
BCP100+CFA	1.16± 0.08###	2.31± 0.06###	2.512± 0.056###	2.02± 0.06###	1.89± 0.118###	1.73± 0.10###	1.62± 0.04###	1.45± 0.09###
BCP300+CFA	1.175± 0.12\$\$	2.23± 0.08\$\$\$	2.42± 0.079\$\$\$	1.917± 0.12\$\$	1.67± 0.141\$\$	1.620± 0.116\$\$\$	1.45± 0.12\$\$\$	1.37± 0.12\$\$\$
ACF10+CFA	1.11± 0.093&&	2.28± 0.086&&&	2.39± 0.082&&&	1.81± 0.17&&&	1.718± 0.134&&&	1.640± 0.149&&&	1.51± 0.09&&&	1.30± 0.114&&&

inflammation from day 1 of administration to day 3, followed by a brief decrease in the inflammatory signs from day 3 to 9 (Figure 2-6).

Effect of Beta-Caryophyllene on change in paw volume

There was a significant increase in paw volume in all Complete Freund's adjuvant (CFA) groups compared to vehicle control. This also had shown a biphasic response where there was a small decrease in paw volume from day 9 to 21. However this change was not significant. The paw volume was maximum on day 3 in all Complete Freund's adjuvant administered rats. On treatment with Beta-Caryophyllene (100 and 300 mg/kg) significantly ($P < 0.01$, $P < 0.001$ respectively) decreased the paw volume, which was observed till end of the study. There was no significant change in paw volume in Beta-Caryophyllene 100 mg/kg treated group.

Effect of Beta-Caryophyllene on Anti-Oxidant Enzymes

Complete Freund's adjuvant rats showed that the levels of nitric oxide and Malondialdehyde were both increased significantly in arthritic animals, whereas the levels of all the endogenous antioxidants [superoxide dismutase and glutathione, catalase] were found to be decreased in arthritic animals. Beta-Caryophyllene significantly reversed the above changes. Beta-Caryophyllene significantly decreased the NO level that of model group. Both Beta-Caryophyllene and Aceclofenac could restore the MDA level to the normal range. Beta-Caryophyllene significantly reduced the level of MDA that of the model group, Although BCP had no regulative effect on the activities of SOD, it could significantly increase the activity of SOD of the model group, and higher than the control group. Treatment with Beta-Caryophyllene increased the activities of GSH that of the model group, whereas Aceclofenac (ACF) had no obvious effect on GSH activity.

Effect of Beta-Caryophyllene on Serum enzyme levels

As a result of inflammation induced by adjuvant, the levels of SGPT, SGOT and ALP were increased in all arthritis rats as compared to control rats. After Beta-Caryophyllene treatment, the levels of these enzymes were significantly decreased in Beta-Caryophyllene 100mg/kg and 300mg/kg rats as compared to control rats. Aceclofenac (10mg/kg) treatment prevented biochemical changes to a greater extent than the Beta-Caryophyllene doses. The SGOT, SGPT, ALP and bilirubin levels of all the groups were evaluated and compared with each other.

Table 3 Effect of Beta-Caryophyllene on Anti-oxidant Enzymes.

Groups	GSH (U/mg protein)	Catalase (U/mgprotein)	SOD (U/min/mg protein)
Group I (Vehicle)	7.745 ± 0.35		1.754 ± 0.16
Group II (CFA)	4.671± 0.60***	0.202± 0.04***	0.418± 0.17***
GroupIII(BCP100mg/kg)	6.589± 0.43##	0.606± 0.11##	1.188± 0.03##
GroupIV(BCP300mg/kg)	8.426± 0.22\$\$\$	0.852± 0.04\$\$\$	1.588± 0.20\$\$\$
GroupV(ACF10mg/kg)	7.468± 0.31&&&	1.060± 0.12&	1.713± 0.16&&

Values are expressed as mean ± SEM of n=6 animals. ***P < 0.001 indicates comparison of negative control group with control group. ###P < 0.05 indicates comparison of low dose group with negative control. \$\$\$P < 0.001, indicates comparison of high dose group with negative control. &&&P < 0.001, &&P < 0.01 indicates comparison of standard with negative control.

Table 4 Effect of Beta-Caryophyllene on Total Protein.

Group	Total Protein (mg/ml protein)
Group I (Vehicle)	9.67± 0.52
Group II (Complete Freund's adjuvant)	5.24± 0.39***
GroupIII (BCP 100mg/kg+ CFA)	7.66± 0.41###
GroupIV (BCP 300mg/kg+CFA)	9.57± 0.44\$\$\$
GroupV (Aceclofenac10mg/kg+CFA)	11.18± 0.51&&&

Values are expressed as mean ± SEM of n=6 animals. ***P < 0.001 indicates comparison of negative control group with control group. ###P < 0.05 indicates comparison of low dose group with negative control. \$\$\$P < 0.001, indicates comparison of high dose group with negative control. &&&P < 0.001, &&P < 0.01 indicates comparison of standard with negative control.

Table 5 Effect of Beta-Caryophyllene on TBARS and Serum Nitrite.

Group	TBARS (nmol/mg protein)	Serum Nitrite (µg/mg protein)
Group I(Vehicle)	1.81± 0.23	3.18± 0.33
Group II(Complete Freund's adjuvant)	5.25± 0.24***	7.33± 0.80***
Group III(BCP 100mg/kg+CFA)	3.75± 0.47##	4.72± 0.35###
Group IV(BCP 300mg/kg+CFA)	2.57± 0.22\$\$\$	3.66± 0.27\$\$\$
Group V(Aceclofenac 10mg/kg+CFA)	1.97± 0.30&&&	3.31± 0.30&&&

Values are expressed as mean ± SEM of n=6 animals. ***P < 0.001 indicates comparison of negative control group with control group. ###P < 0.05 indicates comparison of low dose group with negative control. \$\$\$P < 0.001, indicates comparison of high dose group with negative control. &&&P < 0.001, &&P < 0.01 indicates comparison of standard with negative control.

Table 6 Effect of BCP on Serum SGPT, SGOT Levels.

Groups	SGOT (U/L blood)	SGPT (U/L blood)
Group I (Vehicle)	24.16 ± 1.26	30.16 ± 4.62
Group II (Complete Freund's adjuvant)	68.52± 6.50***	78.72± 4.18***
GroupIII(BCP 100mg/kg+ CFA)	40.51± 8.35###	43.31± 5.95###
GroupIV(BCP 300mg/kg+CFA)	30.53± 4.49\$\$\$	31.50± 5.95\$\$\$
GroupV(Aceclofenac10mg/kg+CFA)	21.74± 3.06&&&	25.43± 4.07&&&

Values are expressed as mean ± SEM of n=6 animals. ***P < 0.001 indicates comparison of negative control group with control group. ###P < 0.05 indicates comparison of low dose group with negative control. \$\$\$P < 0.001, indicates comparison of high dose group with negative control. &&&P < 0.001, &&P < 0.01 indicates comparison of standard with negative control.

Table 7 Effect of BCP on Serum Bilirubin, ALP Levels.

Groups	Bilirubin (U/L blood)	ALP (U/L blood)
Group I(Vehicle)	29.28 ± 5.91	28.16 ± 3.90
Group II(Complete Freund's adjuvant)	74.67± 9.27***	68.67± 7.11***
GroupIII(BCP 100mg/kg+ CFA)	41.29± 6.39###	41.51± 8.21###
GroupIV(BCP 300mg/kg+CFA)	36.83± 5.61\$\$\$	35.43± 3.86\$\$\$
GroupV(Aceclofenac10mg/kg+CFA)	28.53± 2.90&&&	26.53± 3.29&&&

Values are expressed as mean ± SEM of n=6 animals. ***P < 0.001 indicates comparison of negative control group with control group. ###P < 0.05 indicates comparison of low dose group with negative control. \$\$\$P < 0.001, indicates comparison of high dose group with negative control. &&&P < 0.001, &&P < 0.01 indicates comparison of standard with negative control.

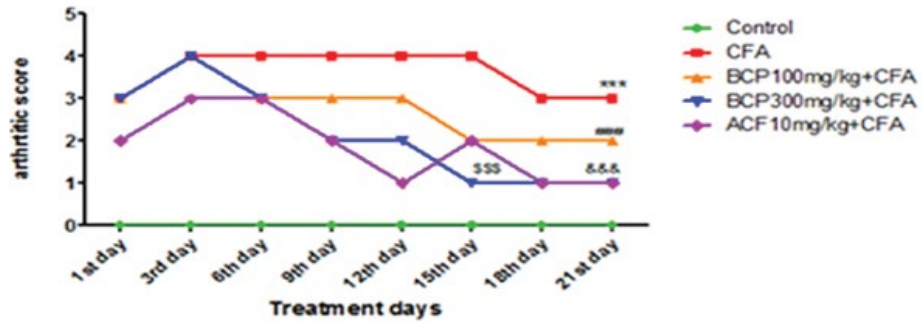


Figure 2 Effect of Beta-Caryophyllene (BCP) on Arthritic index.

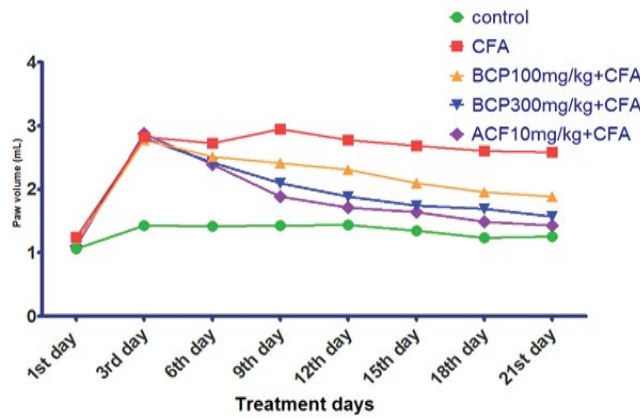


Figure 3 Effect of Beta-Caryophyllene on Paw volume.

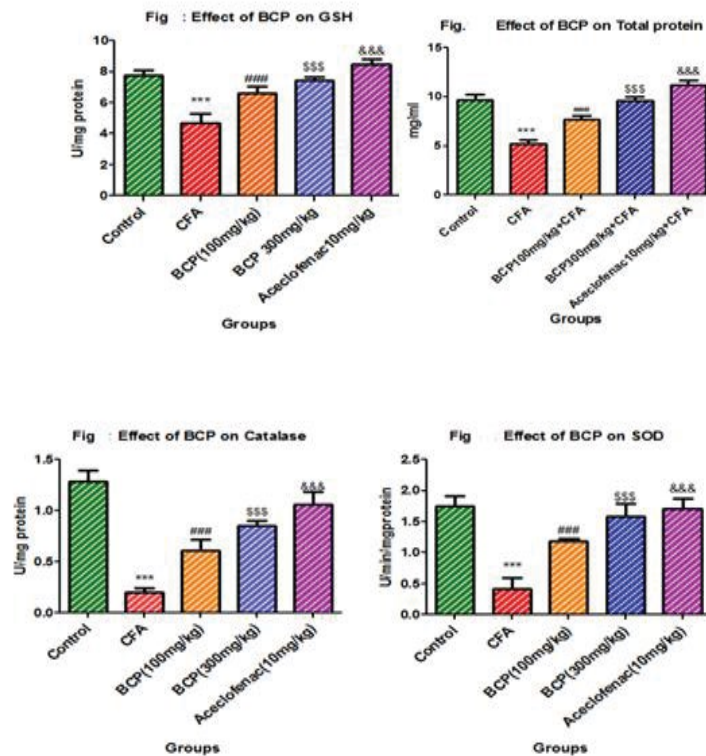


Figure 4 Effect of Beta-Caryophyllene on Anti-oxidant enzymes.

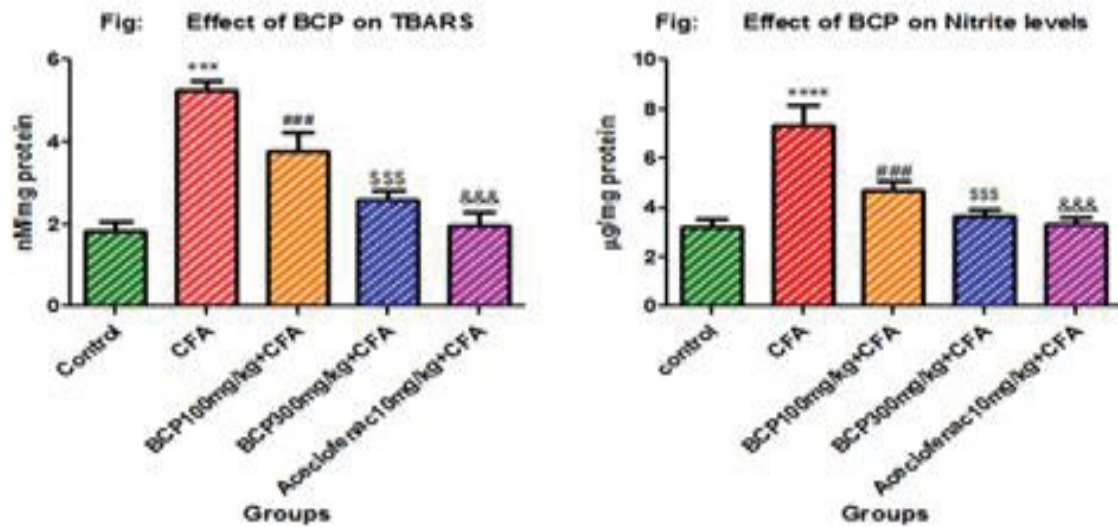


Figure 5 Effect of Beta-Caryophyllene on TBARS and Serum Nitrite.

Radiology of Hind Paws in Adjuvant Induced Arthritic Rat

1. Uninjected control group with no degenerative joint changes
2. Slight soft tissue volume, joint space, subchondral erosion, periostitis, osteolysis, subluxation, and degenerative joint changes.
3. Low to moderate soft tissue volume, joint space, subchondral erosion, periostitis, osteolysis, subluxation, and degenerative joint changes.
4. Pronounced soft tissue volume, joint space, subchondral erosion, periostitis, osteolysis, subluxation, and degenerative joint changes.
5. Excess soft tissue volume, joint space, sub-chondral erosion, periostitis, osteolysis, subluxation, and degenerative joint changes (**Figure 7**).

Histopathology of Proximal Interphalangeal Joints on Adjuvant Induced Arthritic Rats

Control

- Bone appeared normal – Red arrow
- Cartilage between the joints appeared normal – Black arrow
- Synoviocytes surrounding the cartilage appeared normal
- NO pannus formation and no inflammatory cells infiltration noticed (green arrow)

Negative control

- Severe pannus formation (chronic arthritis) surrounding the joints in which extensive proliferation of fibro vascular

tissue or granulation tissue. – Black arrow.

- Extensive accumulation of synovial fluids noticed in between pannus formation – Red arrow

Beta-Caryophyllene 100mg/kg

- Moderate pannus formation in which proliferation of fibro vascular tissue or granulation tissue. – Black arrow
- Also accumulation of synovial fluids in between pannus formation - Red arrow

Beta-Caryophyllene 300mg/kg

- Bone structure appeared normal; no erosion noticed – black arrow
- Cartilage /synovial membranes appeared normal – red arrow
- Mild erosion noticed in the cartilage – green arrow

Aceclofenac 10mg/kg

- Bone and cartilage surrounding the joints appeared normal – black arrow
- Mild pannus formation in which proliferation of fibrous tissue noticed from the cartilage – Red arrow (**Figure 8**).

Discussion

Rheumatoid arthritis is an autoimmune disorder, the immunologically mediated complete Freund's adjuvant induced arthritic model of chronic inflammation is considered as the best available experimental model of rheumatoid arthritis. Complete Freund's adjuvant-induced arthritis is a model of chronic polyarthritis with features that resemble rheumatoid arthritis.

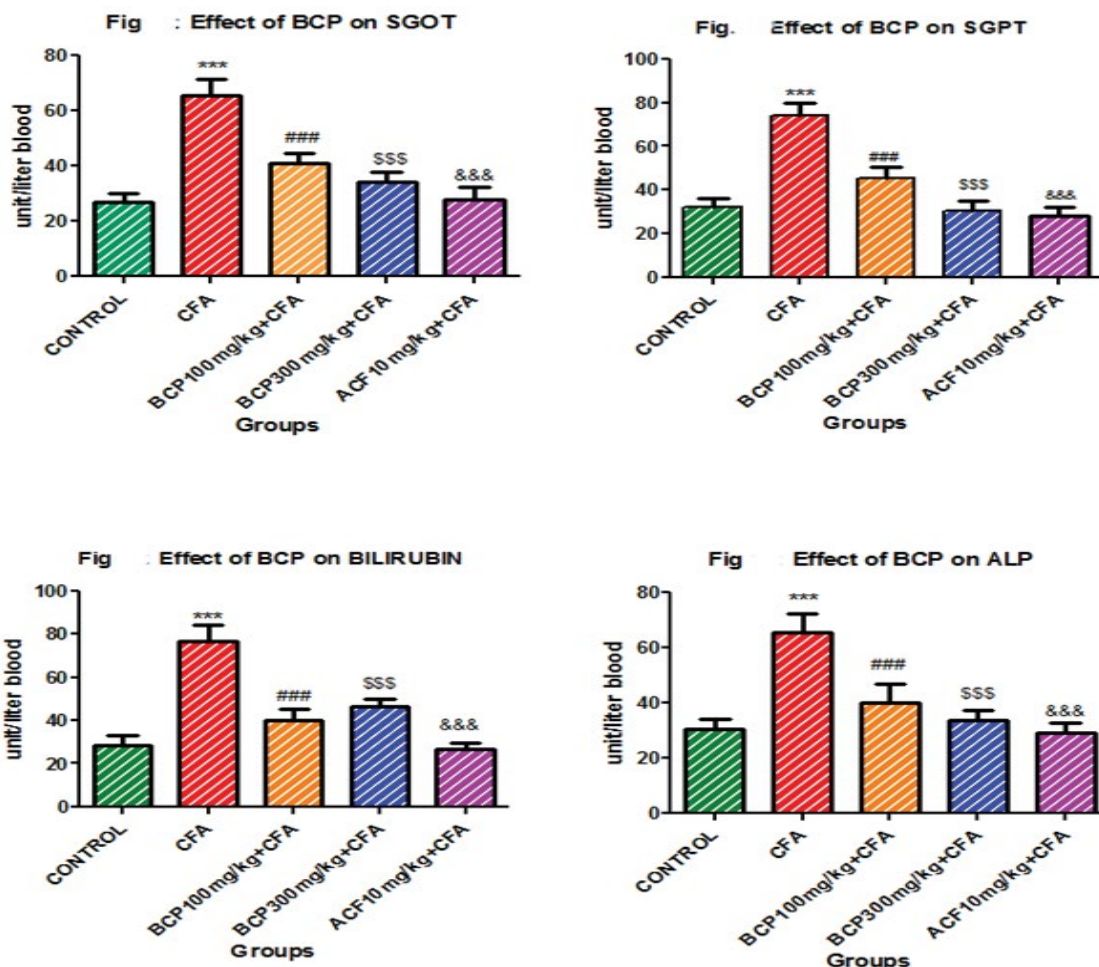


Figure 6 Effect of BCP on Serum SGOT, SGPT, Bilirubin and ALP Levels.

In adjuvant-induced arthritis model, rats developed a chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction and remodeling which have close similarities to human rheumatoid disease. These inflammatory changes ultimately result in the complete destruction of joint integrity and functions in the affected animal. Also, the Complete Freund's adjuvant administered rats showed soft tissue swelling around the ankle joints during the development of arthritis, which was considered as edema of the particular tissues. B-Caryophyllene is a natural bicyclic sesquiterpene and phytocannabinoid receptor agonist which is isolated from clove leaf oil, clove stem oil, cinnamon leaf oil, and pine oil fractions [23].

Paw swelling is an index of measuring the anti-arthritis activity of Beta-Caryophyllene at the dose level 100 & 300mg/kg/p.o. Beta-Caryophyllene administered groups showed marked reduction in paw volume when compared with the arthritic control group (Group II) found that there was significant weight loss, the day following the injection of the adjuvant, but thereafter continued to show normal weight gain in rats. The result of the present study also indicates that there is a close relationship between the extent of inflammation, loss of body weight and arthritic index. [24].

The radical derivatives of oxygen (O₂) are the most important free radicals in the biological systems. The activated O₂ intermediates together with secondarily formed radicals like hydroxyl radicals are able to destroy membrane lipids, proteins, DNA, and cartilage. Neutrophils, macrophages and dendritic cells generate ROS in large amounts in response to activation in CFA induced rats [25]. Moreover, ROS produced by macrophages, lymphocytes and endothelial cells contribute to the destruction of cartilage. Increased lipid peroxidation and decreased enzymatic and non-enzymatic antioxidants are found in Rheumatoid arthritis patients, the MDA levels in Rheumatoid arthritis patients are significantly higher than normal, but the activities of GSH, catalase and SOD are lower. These findings suggest that oxidant stress plays a very important role in the pathogenesis of Rheumatoid arthritis disease. Our study also showed that levels of three oxidation products, MDA and NO, in Complete Freund's adjuvant rats were higher than those in the control, and meanwhile, the activities of antioxidants were lower.

Assessment of the levels of SGOT, SGPT, ALP and bilirubin provides an excellent and simple tool to measure the anti-arthritis activity of the target drug. The activities of these enzymes were significantly increased in arthritic rats. These are good indicators

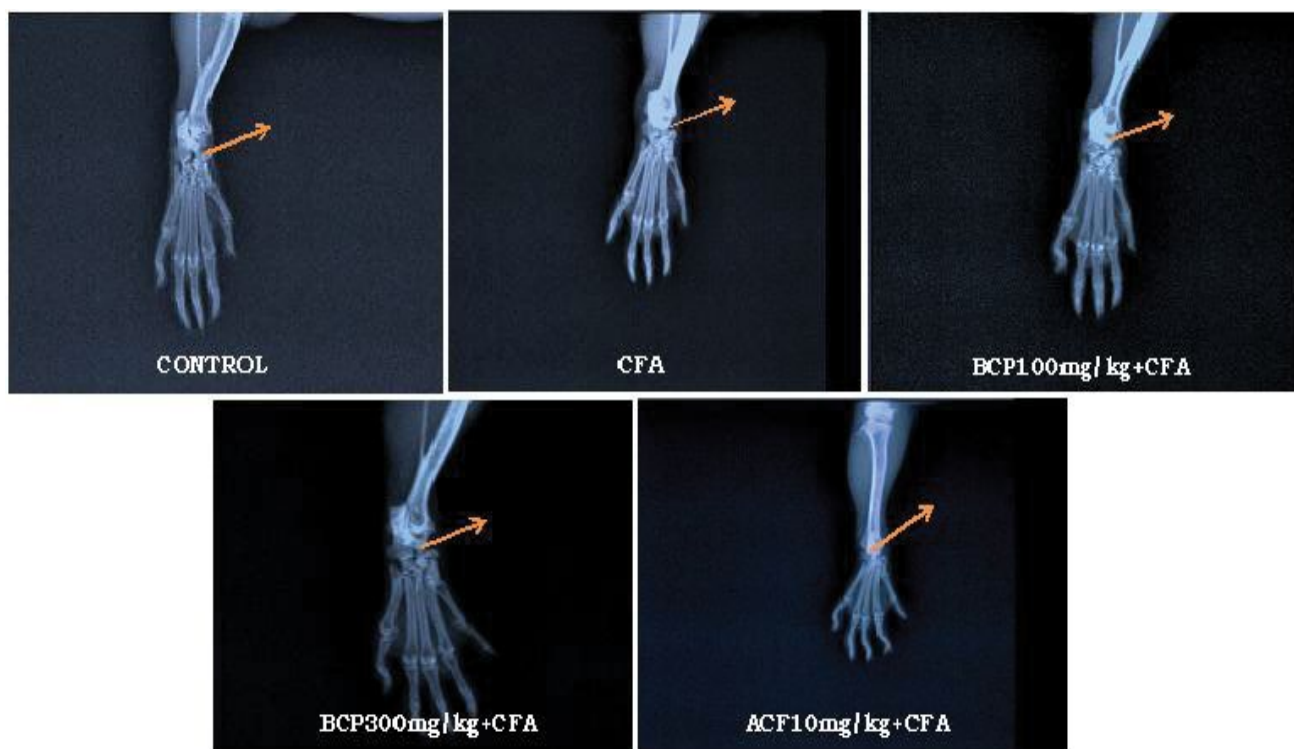


Figure 7 Radiology of Hind Paws in Adjuvant Induced Arthritic Rat.

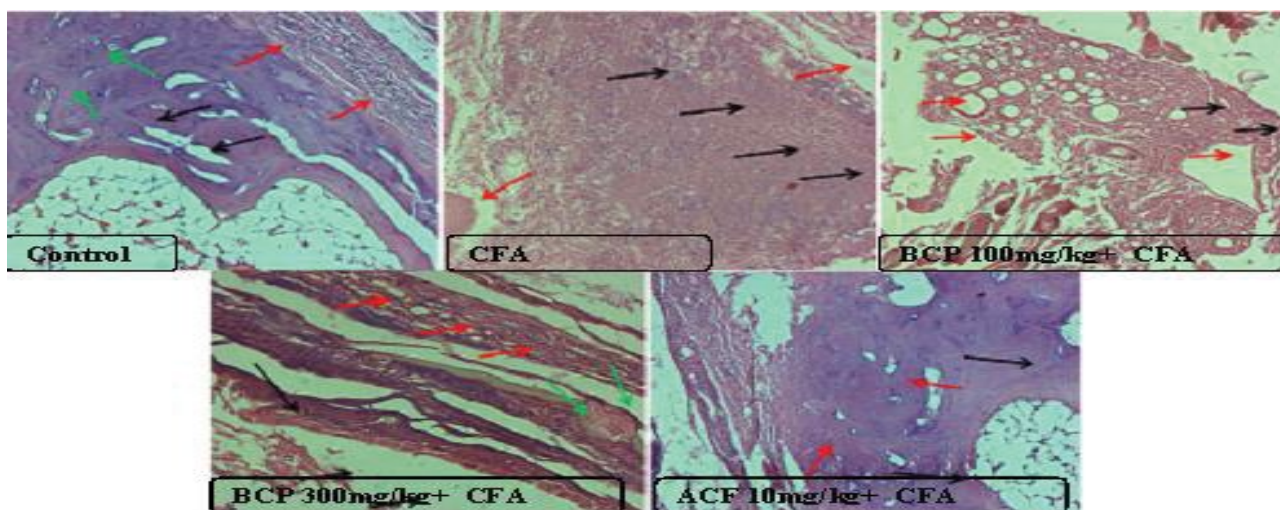


Figure 8 Histopathology of Proximal Interphalangeal Joints on Adjuvant Induced Arthritic Rats.

of liver and spleen impairment, which are also considered to be features of adjuvant arthritis [26]. The result of the present study also indicates that there is a close relationship between control and Beta-Caryophyllene treated groups.

Histopathology provides a noticeable morphological distinctiveness as a practical and unambiguous pathognomonic sign of Rheumatoid arthritis. The histopathological analysis identified the ability of the bones to re-form upon treatment with Beta-Caryophyllene. Bone structures re-calcified upon treatment with the Beta-Caryophyllene dose dependently. The

Beta-Caryophyllene exhibited such therapeutic potential from the study results and is therefore consistent with earlier findings that the ability of a drug to suppress inflammation, synovitis and protect a joint is desired in rheumatoid arthritis therapy.

Radiographic changes in Rheumatoid arthritis conditions are useful diagnostic measures which indicate the severity of the disease. Soft tissue swelling is the earlier radiographic sign, whereas prominent radiographic changes like bony erosions and narrowing of joint spaces can be observed only in the developed stages (final stages) of arthritis [27]. The radiographic features of

the rat joints in adjuvant induced arthritic model are shown in **Figure 2**. In adjuvant induced arthritic rat (group II), soft tissue swelling along with narrowing of the joint spaces were observed which implies the bony destruction in arthritic condition. The standard drug Aceclofenac (10mg/kg) treated groups have prevented this bony destruction and also there is no swelling of the joint. Similar to histopathological studies, Beta-Caryophyllene treatment for 21 days have shown significant prevention against bony destruction by showing less soft tissue swelling and narrowing of joint spaces when compared with Complete Freund's adjuvant control group.

Conclusion

The Beta-Caryophyllene is an effective anti-arthritic agent experimentally and holds prospect in future rheumatoid arthritis treatment. In conclusion, this study has verified that Beta-Caryophyllene suppressed the joint inflammation and destruction in adjuvant arthritic rats. We are confident that our data provide mechanistic evidence for anti-arthritic appliance of Beta-Caryophyllene as a promising candidate for novel therapeutic agent of Rheumatoid arthritis.

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