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Research Article
Anti-arthritic Effect of Baicalein Exert on Complete Freund's Adjuvant-induced Arthritis in Rats by Reducing the Inflammatory Reaction

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Abstract

Background and Objective: Rheumatoid arthritis (RA) is a long-term autoimmune disorder that primarily affected the joints. The aim of the current study was to investigate the anti-arthritic and anti-inflammatory effect of baicalein against complete Freund's adjuvant-induced arthritis via alteration of inflammatory reaction. **Materials and Methods:** Complete Freund adjuvant (CFA) was to induce arthritis in the Wistar rats and rats were divided into a different group and received the oral administration of baicalein until 28 days. The paw edema, body weight and arthritic score were estimated at a regular time interval. At the end of the experimental period, antioxidant, hematological and pro-inflammatory cytokines were estimated. **Results:** Baicalein treated rats showed the down-regulation of paw edema, arthritic score and significantly ($p<0.001$) increased the body weight. Baicalein treatment significantly ($p<0.001$) modulated the antioxidant, hematological parameters and pro-inflammatory cytokines. Baicalein attenuated the effect of CFA on bone surface area, bone volume and trabecular thickness. Baicalein significantly ($p<0.001$) reduced the inflammation, cartilage surface erosion, chondrocyte death and bone erosion. **Conclusion:** Baicalein indicated the beneficial effect against the CFA induced arthritic rats via pro-inflammatory cytokine mechanism.

Key words: Rheumatoid arthritis, baicalein, joint swelling, complete freund adjuvant, bone erosion, pro-inflammatory cytokines, anti-arthritic

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease categorized via joint tenderness, joint swelling and damage of synovial joints[1]. It is also considered as the autoimmune disease, characterized by inflammatory cell proliferation and infiltration of synovial tissue, accompanied by bone destruction and pari-articular cartilage[2,3]. The RA was linked with the extra-articular manifestations especially involving the lung, renal function and heart. The incidence of RA (degenerative disease) as a major cause of disability is about 0.5-1% of the World population and keep increasing with time[4,5]. The ratio of RA incidence in females and males is 1:3. A research suggested that the immunological reaction is an association with genetic factors are thought to be the basis of RA developing[4-6]. The multifarious interaction of different immune modulator (effector cells and cytokines) responsible for joint destruction that starts at the synovial membrane and covers the most intra-articular structure[7,5]. The pathophysiology of RA is still unclear, but few researchers suggested that the inflammatory mediators viz., tumor necrosis factor- α, interleukin-6 and cyclooxygenase-2 played a significant role in the inflammatory reaction of synovial membranes and bone damage observed during RA[8,9]. It was studied that the RA patients synovial membranes infiltrated via granulocyte and lymphocytes that fabricate chronic pro-inflammatory cytokines including TNF-α, IL-1β and IL-6, leading the damage of articular cartilage and start the bone destruction. The activated cells such as T-cell, B-cells and macrophages cells also activate the other cells such as TNF-α, free radicals and degradative enzymes, which in turn can be directed against the antigen in the joint, which further contributed the progression of RA disease and leading to per-articular joint deformation and tissue degradation[10-12]. Generally, etanercept and infliximab (TNF-α inhibitors) most commonly used for the treatment of RA disease[13,14]. Generally, the researcher targeted the TNF-α and IL-6 for the treatment of RA in an animal model and also in human[13,15].

The currently available treatments for RA are disease modifying anti-rheumatic drugs, non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids[16]. The available treatment having a beneficial effect on the RA disease but these treatments having the limitation due to side effects followed prolonged treatment[17,18]. Recently, some alternative therapeutics for RA treatment, targeted the inflammatory cytokines such as infliximab (anti-TNF-α, anakinra (a recombinant human IL-1 receptor antagonist) and etanercept (a recombinant TNF receptor fusion protein) have promising effect in clinical trials and have approved for the RA treatment[13,15]. But the costs of these treatments are too high and show some undesirable side effects. Now, the researchers searching the new therapeutic effect with low cost and low or no side effects drugs. Now, the researcher focusing their research on the plant-based drug to scrutinize the anti-arthritic effects. So the current experimental study was aimed to examine the protective effect of phytoconstituents baicalein against complete Freund's adjuvant-induced arthritis via alteration of pro-inflammatory cytokines.

MATERIAL AND METHODS

Chemicals: Complete Freund adjuvant (CFA), indomethacin and Baicalin were purchased from the Sigma Aldrich, U.S.A. Pro-inflammatory cytokines viz., interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-17 (IL-17) and IgG were purchased from the Abcam Company (Cambridge, U.K.). All other chemical and reagent used in the current experimental study were analytical grade and purchased from the approved vendor.

Experimental animal: For the current experimental study, Swiss Wistar rats (150±5 g, either both sex) were used. The rats were received from the departmental animal house and acclimatization for 1 week before the experimentation protocol. The rats were kept in the standard laboratory condition (22±5°C, 70-85% relative humidity; 12/12 h dark/light circle). The rats have received the standard diet pellet and water *ad libitum*. The current study was approved by the institutional animal ethical committee (JPH/18/11/15). The study was conducted in the month of December, 2018- January, 2019 in China.

CFA induced arthritic model: Single intraperitoneal injection of CFA (0.1 mL containing 10 mg mL^{-1}) was injected into the right hind metatarsal footpad of Wistar rats for inducing arthritis[4,19]. Normal control group rats received the saline in place of CFA (same volume). The first day of immunization was considered as day 0.

Evaluation of arthritis: After successfully induction arthritis, the rats were divided into the 6 groups (normal group added separately) and the rats were grouped as Group I: Received saline (normal control), Group II: Received CFA only (Arthritic control), Group III-V: Received CFA+Baicalein (2.5, 5 and 10 mg kg^{-1}, b.wt.) and Group VI: Received CFA+indomethacin (2.5 mg kg^{-1}, b.wt.), respectively. The rats have received the above treatment for 28 days[4,19].

The body weight and paw edema of all group rats were estimated at a regular time interval. After 28 days the rats were anesthetized and blood samples were collected via puncturing the retro-orbital plexus. After that, the rats were sacrificed excess of anesthesia and organ was used for the estimation of different biochemical parameters[4].

Arthritic index: The arthritic score of all injured rats was estimated via using the previously reported method with minor modification[4]. The scoring system of arthritis was performed on visual scoring. For the scoring, different scale was used such as if unchanged (the score should be 0), if mild swelling and erythema (the score should be 1), if the persist the erythema and moderate swelling (the score should be 2), if persist the erythema and coarse swelling (the score should be 3) and if persist the gross inability and deformity (the score should be 4). The score of the rats more than 1, considered as the arthritic and the score reach maximum up to 16[4].

Antioxidant parameters: The antioxidant parameters such as lipid peroxidation (LPO), glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were estimated via using the Kits followed the instruction of manufactures.

Hematological parameters: Hematological parameters such as red blood cells (RBC), white blood cells (WBC), erythrocytes sedimentation rate (ESR) and hemoglobin (Hb) were estimated via using the previously reported method with minor modification[4,20].

Spleen and thymus index: On the end of the experimental protocol (day 28), all group rats were sacrificed and spleen and thymus successfully removed from the rats and weighted. The Spleen and thymus indexed were calculated as the ration (mg g^{-1}) of spleen and thymus wet weight vs body weight, respectively[21,22].

Micro-CT evaluation: Briefly, the right hind paw and joints were scanned and prepared the 3D structure using the Viva CT 40 Micro-CT. for the quantitative estimation, the level of bone changes, the following parameters such as trabecular thickness, bone surface area and bone volume were estimated[23,24].

Pro-inflammatory cytokines: Pro-inflammatory cytokines such as TNF-α, IL-1β, IL-2, IL-6, IL-10, IL-12, IL-17 and IgG were estimated as per the instruction of manufacture instruction.

Histology severity: Histological severity was estimation via using the following parameters such as cartilage surface erosion, joint inflammation, bone erosion and chondrocyte death[25,26].

Statistical analysis: All the result presented as Mean±SEM with at least three experiments. While the data were compared by one-way ANOVA followed by Dennett's test. A $p<0.05$ was considered as the statistical significance.

RESULTS

Effect of baicalein on paw edema: The CFA induced control group rats exhibited increased paw edema in a complete experimental study. Normal control group rats did now show any sign and symptom of increase paw edema. The CFA induced arthritic rats showed the maximum value for paw edema at 21 days after the induction of arthritis. Dose-dependent treatment of baicalein significantly reduced the paw edema at a dose of 2.5, 5 and 10 mg kg^{-1}. Indomethacin treated group rats showed the reduction of paw edema, respectively (Table 1).

During arthritis, the arthritic score was considerably increased and confirm the disease progression. A similar effect was observed in the CFA induced arthritic group rats. The CFA induced group rats showed the maximum arthritic score on day 21. Baicalein significantly decreased the arthritic score at a dose of 2.5, 5 and 10 mg kg^{-1}. On the country, indomethacin treated group rats showed a decreased arthritic score (Table 2).

Effect of baicalein on body weight: The CFA induced arthritic group rats showed the initial body weight. The CFA induced arthritic rats demonstrated decreased body weight at the end of the experimental study and baicalein showed the increased body weight at a dose level of 2.5, 5 and 10 mg kg^{-1}. Indomethacin treated group rats exhibited an increased body weight at the end of the experimental study (Table 3).

Effect of baicalein on spleen and thymus index: The CFA induced arthritic rats showed the increased spleen index and thymus index and dose-dependently treatment of baicalein significantly ($p<0.001$) reduced the spleen index and thymus index. On the other hand, indomethacin treated group rats showed the reduction of spleen index and thymus index (Fig. 1).

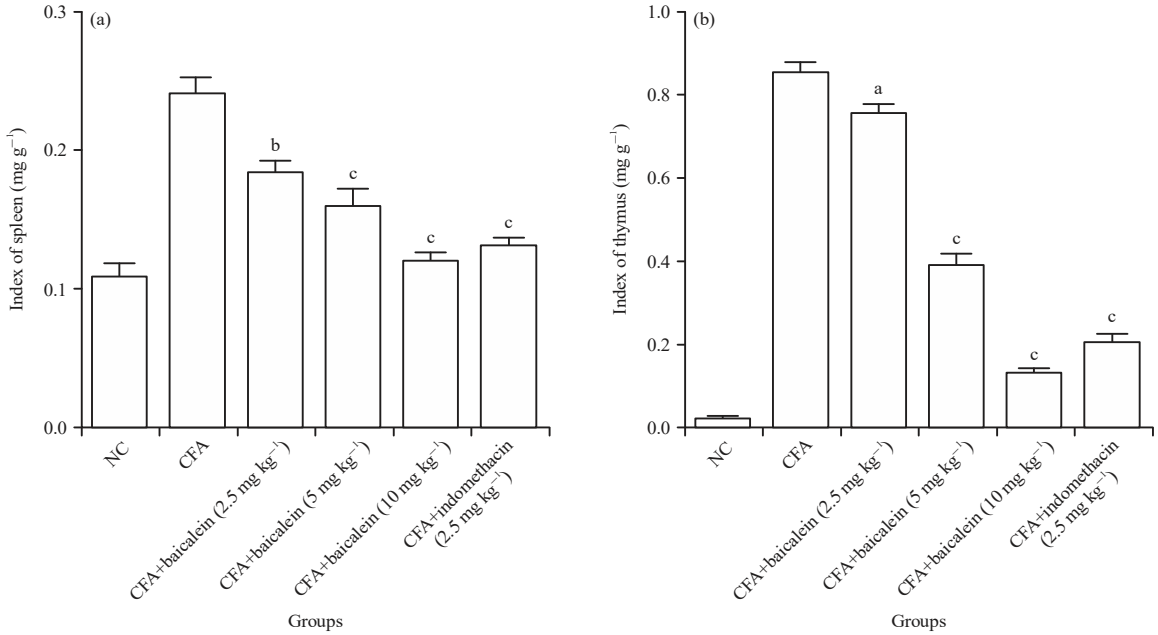

Fig. 1(a-b): Effect of baicalein on the index of spleen and thymus of CFA induced arthritis rats. (a) Index of spleen and (b) Index of thymus

CFA: Complete freund's adjuvant, NC: Normal control, Data are presented as the Mean±SEM, [a]p<0.05, [b]p<0.01, [c]p<0.001 compared with the cont

Table 1: Effect of baicalein on the paw swelling on CFA induced arthritis rats

	Days (Increase the paw edema)				
Groups	14	17	21	25	28
CFA	0.61±0.03	0.89±0.07	1.23±0.02	1.04±0.04	0.95±0.05
CFA+baicalein (2.5 mg kg^{-1})	0.60±0.05^{ns}	0.82±0.05*	1.11±0.02**	0.89±0.05**	0.66±0.05***
CFA+baicalein (5 mg kg^{-1})	0.58±0.06*	0.80±0.06**	1.06±0.03***	0.82±0.02***	0.58±0.02***
CFA+baicalein (10 mg kg^{-1})	0.55±0.04**	0.75±0.02***	0.90±0.05***	0.70±0.04***	0.40±0.04***
CFA+indomethacin (2.5 mg kg^{-1})	0.56±0.02*	0.77±0.08***	0.91±0.03***	0.72±0.03***	0.46±0.03***

CFA: Complete freund's adjuvant, Data are presented as the Mean±SEM, *p<0.05, **p<0.01, ***p<0.001 compared with the cont

Table 2: Effect of baicalein on the arthritic index on CFA induced arthritis rats

	Arthritic score				
Groups	14	17	21	25	28
CFA	5.7±0.34	7.4±0.65	10.5±1.23	9.95±1.01	9.00±0.93
CFA+baicalein (2.5 mg kg^{-1})	5.5±0.65^{ns}	5.7±0.76**	9.85±0.73*	8.75±0.93**	8.05±0.67**
CFA+baicalein (5 mg kg^{-1})	5.2±0.83*	5.5±0.74***	9.65±0.93**	8.55±0.89***	7.75±0.43***
CFA+baicalein (10 mg kg^{-1})	4.7±0.34**	5.3±0.84***	8.25±0.78***	6.70±0.73***	3.85±0.32***
CFA+indomethacin (2.5 mg kg^{-1})	4.9±0.35**	5.4±0.93***	8.40±0.83***	6.80±0.81***	4.05±0.41***

CFA: Complete freund's adjuvant, Data are presented as the Mean±SEM, *p<0.05, **p<0.01, ***p<0.001 compared with the cont

Table 3: Effect of baicalein on the body weight on CFA induced arthritis rats

	Body weight (g)					
Groups	0	5	7	14	21	28
Normal control	150.0±4.56	157.0±3.45	159.0±4.09	177.65±3.04	187.30±3.45	200.02±4.32
CFA	156.0±6.34	158.0±4.56	157.0±3.45	149.30±4.21	142.04±5.64	138.45±3.45
CFA+baicalein (2.5 mg kg^{-1})	155.0±5.43^{ns}	158.7±6.54^{ns}	159.0±5.43*	164.00±5.12***	168.40±4.12***	175.40±5.12***
CFA+baicalein (5 mg kg^{-1})	158.0±5.89^{ns}	161.7±5.06*	164.3±4.57**	168.03±4.09***	176.30±6.43***	186.40±5.37***
CFA+baicalein (10 mg kg^{-1})	159.0±3.23^{ns}	164.0±4.95**	166.3±5.43***	177.80±5.12***	185.40±3.48***	196.84±4.52***
CFA+indomethacin (2.5 mg kg^{-1})	159.5±5.68^{ns}	163.0±5.04**	165.3±4.56***	174.50±4.09***	184.30±4.05***	194.53±4.58***

CFA: Complete freund's adjuvant, Data are presented as the Mean±SEM, *p<0.05, **p<0.01, ***p<0.001 compared with the cont

Effect of baicalein on bone destruction: The CFA rats exhibited the markedly joint destruction by affecting the bone volume, bone surface area and trabecular thickness and baicalein significantly reduced the joint destruction in a dose-dependent manner by attenuating the effect of CFA (Table 4).

Effect of bone parameters: The bone parameters such as erosion, inflammation, cartilage surface erosion and chondrocyte death of all group rats were presented in Fig. 2. Normal control group rats did not show any sign and symptom of bone deformity. The CFA induced arthritic rats exhibited the increased level of erosion, inflammation, cartilage surface erosion and chondrocyte death and dose-dependent treatment of baicalein significantly reduced the erosion, inflammation, cartilage surface erosion and chondrocyte death at dose-dependent manner.

Effect of baicalein on the hematological parameter: The CFA induced arthritic rats exhibited the increased level of WBC, ESR and reduced level of RBC, Hb and baicalein treatment significantly ($p<0.001$) decreased the WBC, ESR and increased the level of RBC, Hb (Fig. 3). A similar effect was observed in the indomethacin-treated group.

Table 4: Effect of baicalein on the bone severity of CFA induced arthritis rats

	Bone severity		
Groups	Bone volume (mm^3)	Bone surface area/bone volume (mm^{-1})	Trabecular thickness (mm)
Normal control	21.76±2.34	12.12±2.93	0.21±0.05
CFA	9.34±3.21	21.34±3.45	0.08±0.02
CFA+baicalein (2.5 mg kg^{-1})	11.87±2.65*	17.87±4.34*	0.10±0.06*
CFA+baicalein (5 mg kg^{-1})	13.34±3.04**	15.45±2.36**	0.14±0.04**
CFA+baicalein (10 mg kg^{-1})	17.45±3.12***	12.04±3.85***	0.18±0.06***
CFA+indomethacin (2.5 mg kg^{-1})	15.65±1.45***	13.04±2.34***	0.16±0.3***

CFA: Complete freund's adjuvant, Data are presented as the Mean±SEM, *p<0.05, **p<0.01, ***p<0.001 compared with the cont

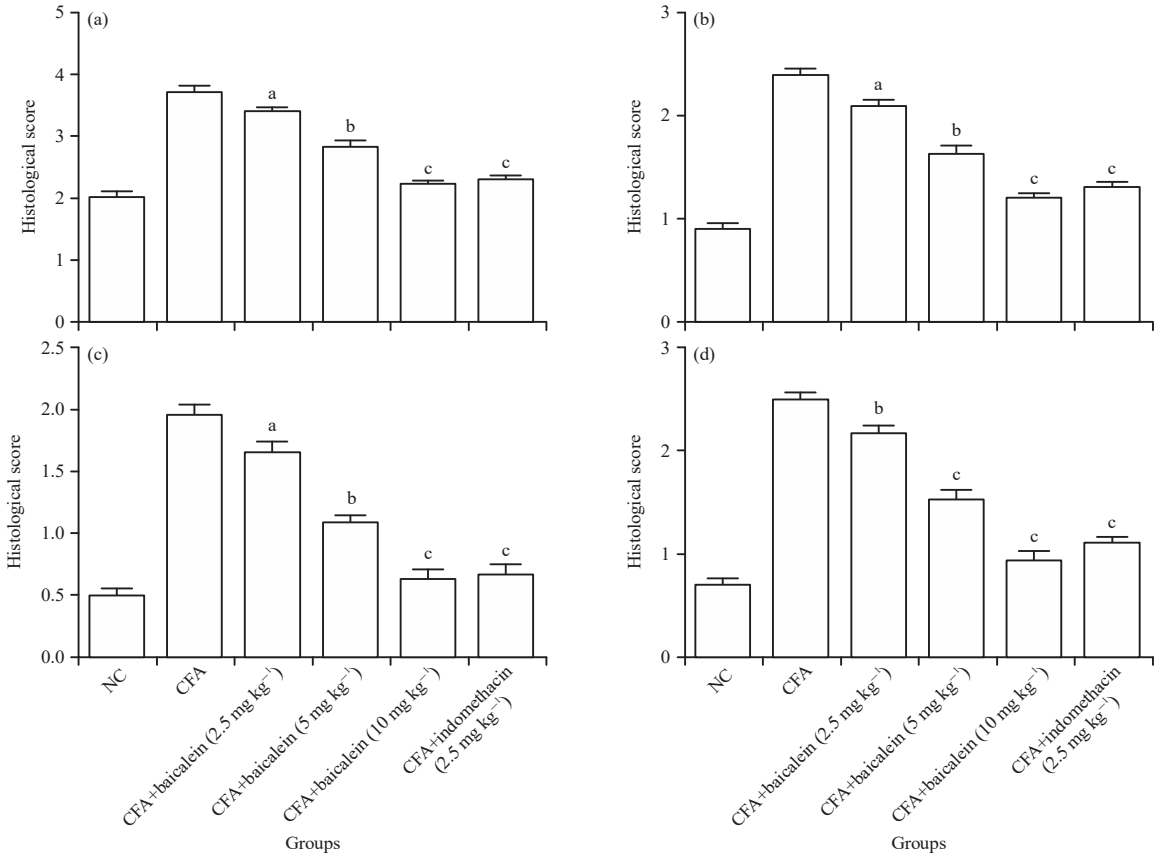

Fig. 2(a-d): Effect of baicalein on the bone parameters of CFA induced arthritis rats. (a) Inflammation, (b) Chondrocytes death, (c) Cartilage surface erosion and (d) Bone erosion

CFA: Complete freund's adjuvant, NC: Normal control, Data are presented as the Mean±SEM, [a]p<0.05, [b]p<0.01, [c]p<0.001 compared with the cont

Effect of baicalein on antioxidant parameter: The CFA induced arthritic rats exhibited the increased level of LPO and reduced level of GSH, SOD, GPx and baicalein treatment significantly (P<0.001) decreased the LPO and increased the level of GSH, SOD, GPx (Fig. 4). Indomethacin treated group showed the reduced LPO and increased the level of GSH, SOD, GPx, respectively.

Effect of baicalein on pro-inflammatory cytokines: The CFA induced group rats showed the increased level of TNF-α, IL-1β, IL-2, IL-6, IL-12, IL-17, IgG and reduced level of IL-10. The CFA induced group rats treated with baicalein and indomethacin exhibited the reduced level of TNF-α, IL-1β, IL-2, IL-6, IL-12, IL-17, IgG and increased level of IL-10 (Fig. 5, 6).

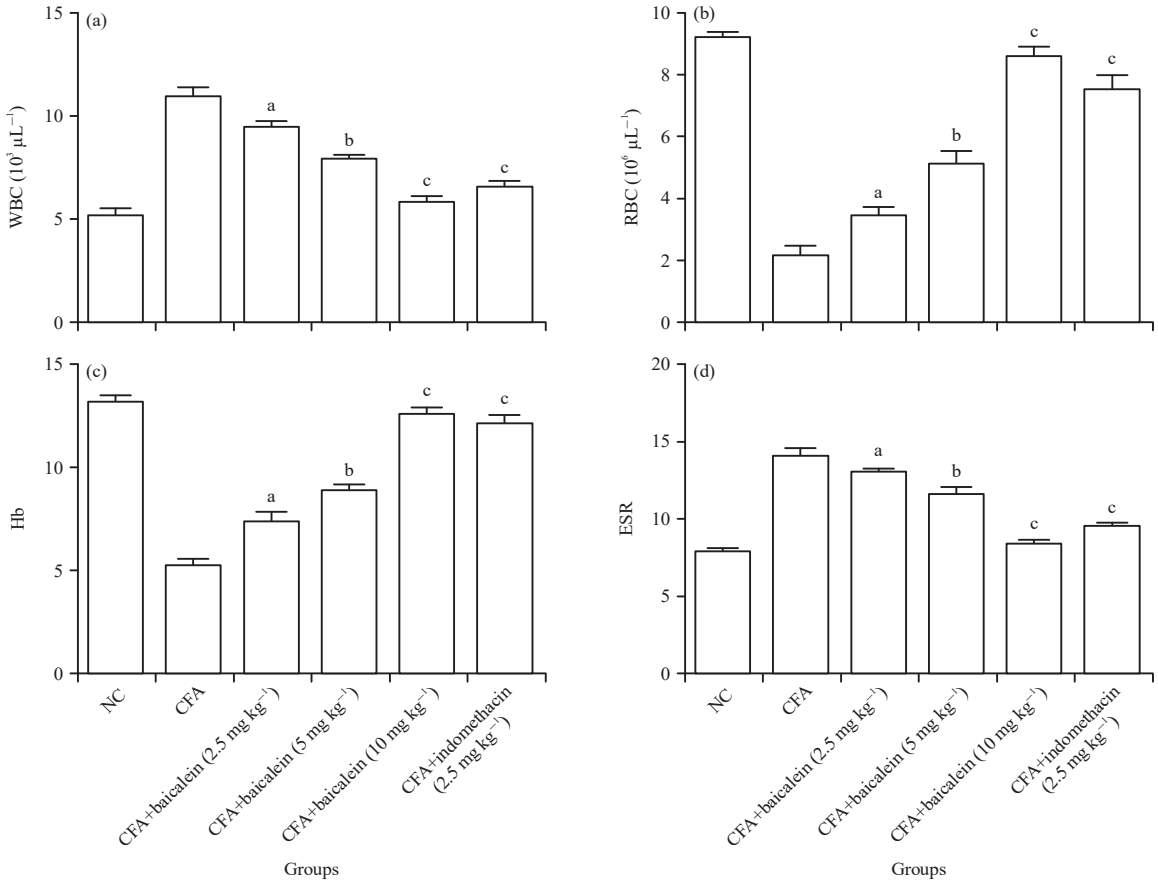

Fig. 3(a-d): Effect of baicalein on the haematological parameters of CFA induced arthritis rats. (a) WBC, (b) RBC, (c) Hb and (d) ESR

CFA: Complete freund's adjuvant, NC: Normal control, Data are presented as the Mean±SEM, [a]p<0.05, [b]p<0.01, [c]p<0.001 compared with the cont

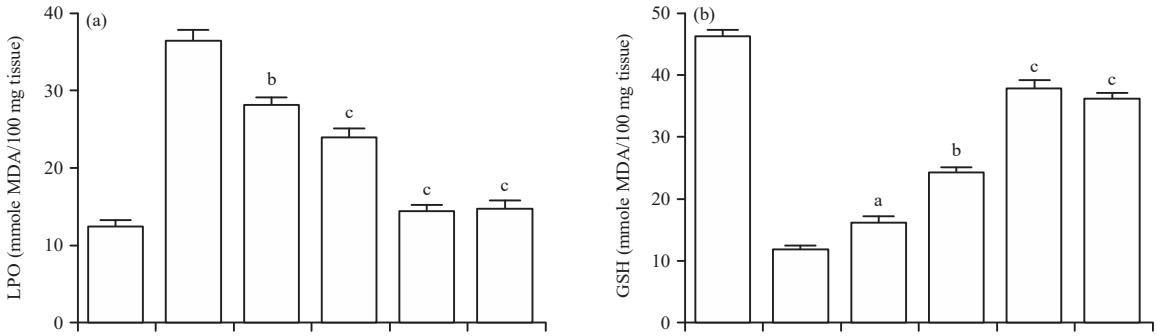

Fig. 4(a-d): Continue

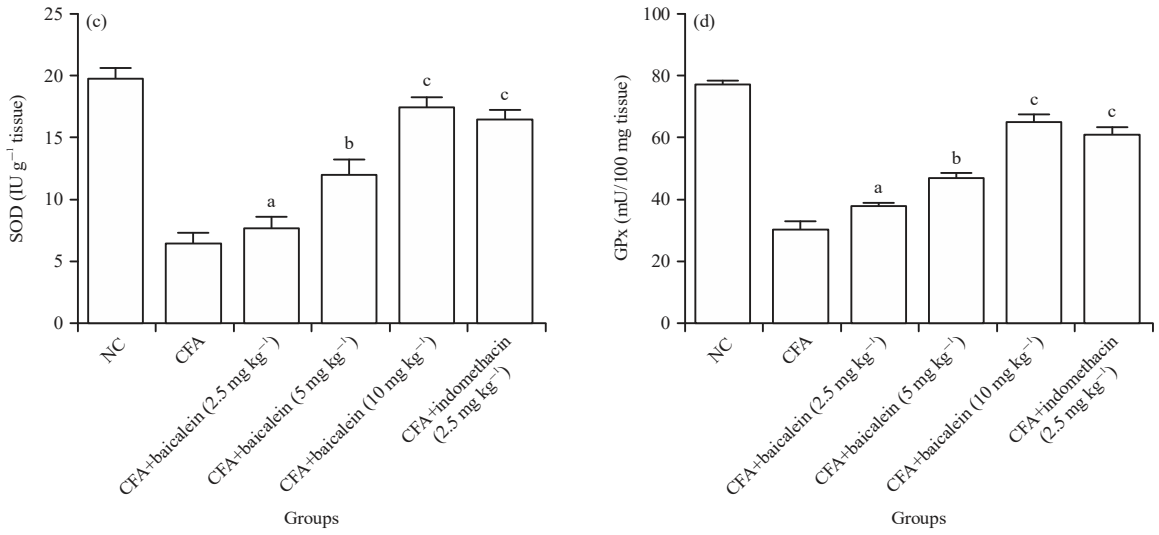

Fig. 4(a-d): Effect of baicalein on the antioxidant parameters of CFA induced arthritis rats. (a) LPO, (b) GSH, (c) SOD and (d) Gpx

CFA: Complete freund's adjuvant, NC: Normal control, Data are presented as the Mean±SEM, [a]p<0.05, [b]p<0.01, [c]p<0.001 compared with the cont

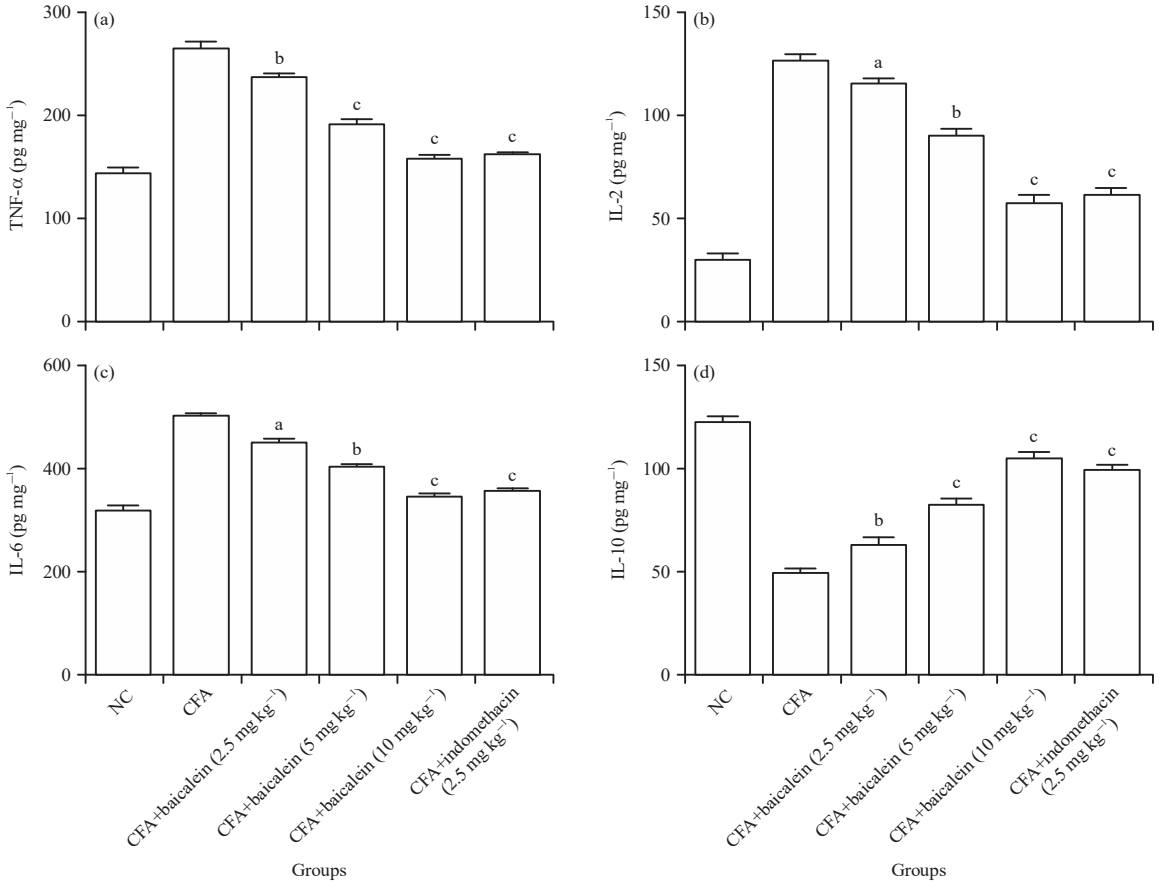

Fig. 5(a-d): Effect of baicalein on the pro-inflammatory cytokines of CFA induced arthritis rats. (a) TNF-α, (b) IL-2, (c) IL-6 and (d) IL-10

CFA: Complete freund's adjuvant, NC: Normal control, Data are presented as the Mean±SEM, [a]p<0.05, [b]p<0.01, [c]p<0.001 compared with the cont

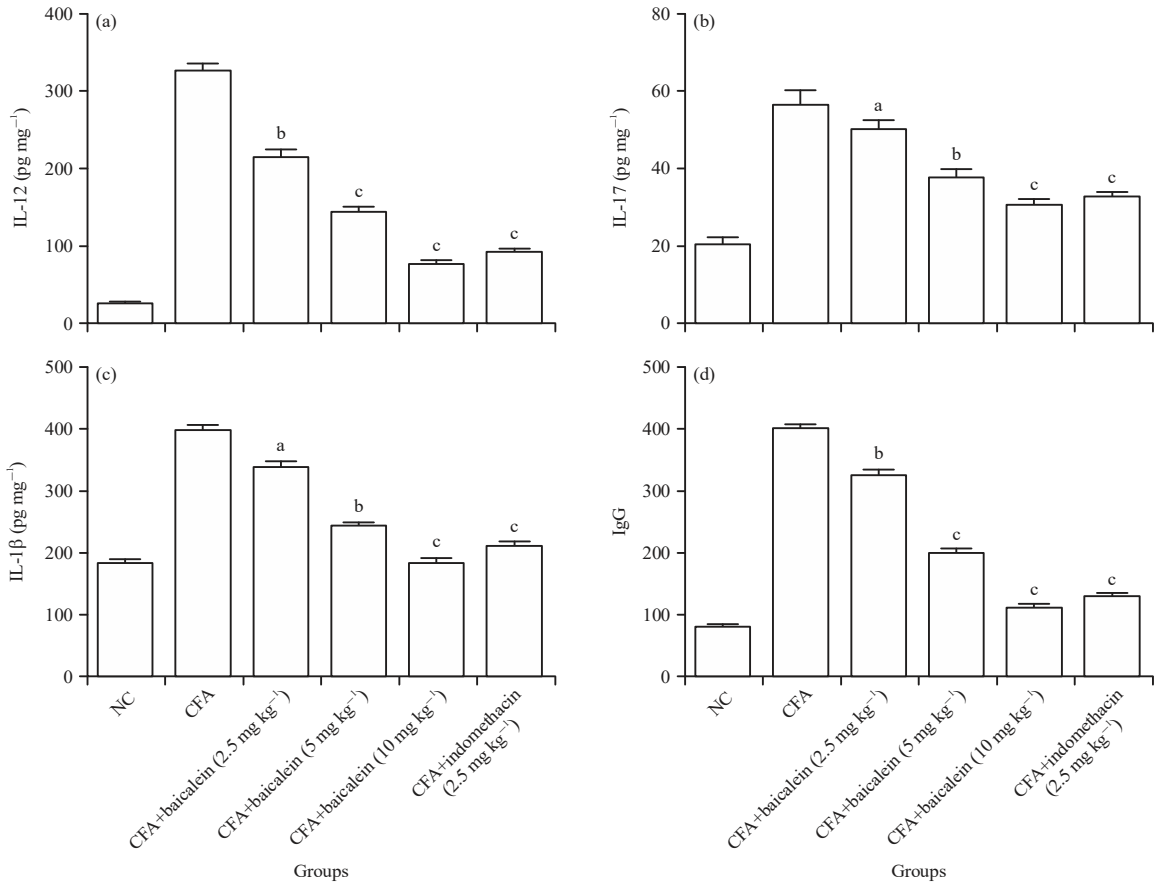

Fig. 6(a-d): Effect of baicalein on the pro-inflammatory cytokines of CFA induced arthritis rats. (a) IL-12, (b) IL-17, (c) IL-1β and (d) IgG

CFA: Complete freund's adjuvant, NC: Normal control, Data are presented as the Mean±SEM, $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ compared with the cont

DISCUSSION

The RA is a complex autoimmune disease mediated inflammatory reaction which attacks to the soft tissue of joint and leads to the joint pain, inflammation and swelling[27,28]. Various factors concerned in the expansion of the RA disease and the RA patients usually required the very long treatment with medications[29,30]. The CFA induced animal model characterized by cartilage destruction, arthrocele, bone erosion, synovitis and generally considered an adequate representation of human RA pathogenesis[31,32]. In the current experimental study, CFA induced model was used to scrutinize the anti-arthritic and anti-inflammatory of baicalein for the treatment of arthritis-related bone damage. The previous investigation suggested that the hematological parameters play an important role in the pathophysiology of RA[33,34]. It was studied that WBC considered as the significant marker of the immune system which linked to the induction of the inflammation and its related other infectious diseases[33,35]. In RA infection the secretion of pro-inflammatory cytokines is started into the injured area and level of WBC in the circulation is increased. It also produces the inflammatory reaction, stimulate the colony growth and macrophages[36]. On the other hand, reduction level of RBC and Hb, showed the down-regulation of erythropoietin and destruction of premature RBCs. Other hematological parameters such as ESR produces the endogenous proteins such as fibrinogen and α/β globulin, which further extend the RA disease[4,36]. During the arthritic condition, CFA induced group rats exhibited the reduced level of RBC, Hb and increased the level of WBC, ESR and baicalein treatment significantly reduced the WBC, ESR level and increased the level of RBC, Hb and exhibited the anti-arthritic and immune modulating effects.

The findings of current study was in agreement with previous researched which stated that the hepatic LPO was considerably increased in CFA induced arthritic rats and the activity of SOD, GSH and GPx were considerably decreased[37-44]. It was also declared that the inflammatory reaction also boosted during the alteration the antioxidant defense[39-41]. LPO, generated during the generation of oxidative stress, which may start the degradation the cell membrane damage[41].

It was studied that the pro-inflammatory cytokines from inflammatory cells and synovium, lymphocytes and monocytes playing an important role in the initiation of RA disease[1,45,46]. In the current experimental study, the pro-inflammatory cytokines such as IL-2 and IL-12 were considerably up-regulated and IL-10 was significantly decreased in the CFA induced arthritic rats. Previous research indicated that the pro-inflammatory cytokines may play a significant role in the RA pathophysiology of human and animal models. It was examined that the IL-12 showed the synergizing effect with various cytokines and induces the generation of pro-inflammatory cytokines[46-48]. Other anti-inflammatory cytokines including IL-4 and IL-10, boosted during the inflammatory disease[49,50].

Previous research suggested that the inflammatory reaction closely connected to the RA pathogenesis[1,45]. Researchers stated that the pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 play a significant role in the initiation and expansion of RA[51,52]. Now, the researcher focused on targeting the pro-inflammatory cytokines to treat arthritis. Pro-inflammatory cytokines such as TNF-α targeted for the treatment of RA[53,54]. In the current study, TNF-α, IL-1β and IgG level was higher observed in the CFA induced group of rats as compared to other group rats. The CFA induced group rats treated with baicalein showed the decreased level of TNF-α, IL-1β and IgG.

CONCLUSION

Collectively, on the basis of the result, baicalein successfully reduced the paw swelling, arthritis and increase the body weight. During arthritis, spleen and thymus index considerably increased and baicalein reduced the spleen and thymus index. Baicalein also attenuate the effect of CFA on bone surface area, bone volume and trabecular thickness. It reduced the pro-inflammatory cytokines and inflammatory mediators. The results of the current experimental study indicated the beneficial effect of baicalein against the CFA induced arthritic rats via pro-inflammatory cytokine mechanism.

SIGNIFICANCE STATEMENT

This study discovers the beneficial effects of baicalein on Complete Freund's Adjuvant-Induced Arthritis induced arthritic rats via pro-inflammatory cytokines pathway. Further, this study will help the researcher to uncover the critical areas of arthritis that many researchers were not able to explore. Thus a new theory on arthritis may be arrived at.

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