IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

IN-VIVO ANTI-ARTHRITIC EFFECT OF COMBINATION OF BOSWELLIC ACID, BERBERINE AND SILYMARIN IN FREUND' S COMPLETE ADJUVANT INDUCED ARTHRTIS IN RATS

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ABSTRACT: Rheumatoid arthritis is a chronic autoimmune disease, which affects approximately 1% adult population. Boswellic acid, berberine, silymarin are major constituent of *boswellia serrata, berberies aristata and milk thistle* respectively and are reported to be effective as anti-inflammatory and anti-arthritic activity. The present study was undertaken to evaluate anti-arthritic activity of combination of boswellic acid, berberine, silymarin in FCA-induced arthritic rats. BBS (150 mg/kg bw p.o) was administered for 28 days and various physical (body weight, paw volume and paw thickness), haematological, biochemical and histopathological parameters were assessed. The BBS showed a significant ($P<0.001$) reduction in paw volume and paw thickness from the 14th to 28th day in the developing phase and reversed the haematological and biochemical parameters as compared to arthritic rats. Histological reports also suggested that histological changes reversed in BBS treated rats. BBS treatment in developed phase has shown moderate anti-arthritic effect. Present study indicated that BBS formulation has significant anti-arthritic activity and beneficial in early stage of arthritis and moderate effect in late stages. Further studies are needed to explore the underlying mechanism in molecular level.

Index Terms - Boswellic acid, berberine, silymarin and Freund's complete adjuvant

1. INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune chronic inflammatory disease. Characterized by hyperplasia of synovial membrane, infiltration of inflammatory cells, neovascularization, cartilage erosion and joint destruction(Perumal, Ekambaram, and Dhanam 2017)(Chunxia et al. 2011). It mainly affects joints and multiple joints at the same time(Yen-Ju Lin 1, Martina Anzaghe 2,† and Stefan Schülke 1,* n.d.)(Scherer, Häupl, and Burmester 2020). RA may proceed to severe impairment, with direct negative effects on lifestyle and a rise in mortality rate(Gomes et al. 2013)(Mahdi et al. 2018). The prevalence of RA is about 0.5–1.0% of the general population(Almutairi et al. 2020). Persons of all ages are susceptible to developing RA, although the prevalence has increased significantly in people over the age of 40 years, particularly women, who are two to three times more susceptible than men(Mahdi et al. 2018)(Yen-Ju Lin 1, Martina Anzaghe 2,† and Stefan Schülke 1,* n.d.).

The imbalance between pro-inflammatory and endogenous anti-inflammatory mediators causes synovial membrane inflammation and joint destruction in RA. Interleukins (IL-1, IL-1, IL-6, IL-18, and IL-20), tumor necrosis factor (TNF)-, C reactive protein (CRP), monocyte chemo attractant protein-1 (MCP-1), receptor activator of nuclear factor-kB ligand (RANKL) fractalkine, matrix metalloproteinase-9 (MMP-9) and adhesion molecules all contribute to the development of RA , joint and cartilage destruction(Yen-Ju Lin 1, Martina Anzaghe 2,† and Stefan Schülke 1,* n.d.)(Scherer, Häupl, and Burmester 2020). Inflammation in joints is by complex interplay between different dendritic cell (DC) subtypes, T cells, B cells, macrophages, neutrophils, fibroblasts and osteoclasts(Yen-Ju Lin 1, Martina Anzaghe 2,† and Stefan Schülke 1,* n.d.).

Presently rheumatoid arthritis is treated with a wide variety of medicines such as disease modifying anti-rheumatoid drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), and corticosteroids help in reducing inflammation of the joint(s) and decreasing pain but they are accompanied by serious adverse effects (Dragos et al. n.d.). So, there is a dire need to find safe drug for RA with minimal adverse effects that can be used for long term treatment.

The medicinal plant based drugs are favored over conventional medicines by patients because of decreased mobility, fear of surgery, ever-growing medicinal cost and adverse effects of novel drugs. Herbal remedies diminish the manifestations of illness and increase the quality of life. Therefore, there is a tremendous increase in the use of plant based health products in developing and developed countries.

From the literature survey, it is evident that boswellic acid, berberine and silymarin individually have anti-inflammatory and antiarthritic activity. Boswellic acid, pentacyclic triterpenes, obtained from different Boswellia species(Iram, Khan, and Husain 2017). It has reported many beneficial activities against arthritis, inflammation, asthma, cancer, hyperlipidaemia, autoimmune encephalitis, chronic inflammatory bowel diseases etc(Roy et al. 2019). Berberine, quaternary ammonium salt from the group of isoquinoline alkoloid, isolated from roots rhizomes and stem bark of various medicinal plants from the Ranunculaceae, Rutaceae and Berberidaceae families(Cicero and Baggioni 2016). It has many medicinal properties in cancer, digestive, metabolic, cardiovascular, neurological diseases, anti-inflammatory and antiarthritic activity etc(Gaba et al. 2021). Silymarin, mixture of flavono ligans comprieses mainly three isomers silybin, sildianin and silychristin, isolated from the fruit of *milk thistle (silybum marrinum)*(Gupta et al. 1978)(Saber et al. 2020). It has hepatoprotective, anti-inflammatory, antiarthritic, anticancer, immunomodulatory, neuroprotective activity etc(Veronika 2020).

Therefore, the present study is undertaken to evaluate in-vivo anti-arthritic activity of formulation containing boswellic acid, berberine and silymarin in FCA-induced arthritic rats.

1. Material and methods:

2.1 Drugs and chemicals:

Boswellic acid, berberine and silymarin were purchased from Yucca enterprises Mumbai. Freund's complete adjuvant was purchased from Sigma Aldrich. The diagnostic assay kits such as alkaline phosphatase (ALP), Aspartate Transaminase (AST), Alanine Transaminase (ALT) and C-reactive protein (CRP) were purchased from Unitron Bio Medicals Bangalore. All other chemicals used in the study were of analytical grade.

2.2 Experimental animals:

Male wistar albino rats weighing 150–200g were procured from CPCSEA registered breeder Sri Raghavendra enterprises, (841/PO/Bt/S/04/CPCSEA) were used in the study. The study has been approved by Institutional Animal Ethical Committee, Nargund College of Pharmacy (1051//Re/S/S/CPCSEA). Animals were housed in polypropylene cages with paddy husk as a bedding material, which was changed thrice a week. They were maintained under standard environmental conditions (temperature 22 ± 2˚C; relative humidity60±5% and 12 h light/dark cycles;) and were fed with standard pellet diet and distilled water ad libitum. The procedures performed in the study were in accordance to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. The biomedical disposal was sent to Maridi Bio industries Pvt Ltd, Bangalore.

2.3 Acute oral toxicity study:

An acute oral toxicity study for formulation containing BBS was carried out in accordance with OECD guidelines 425 A 5000 mg/kg BBS oral test dose was administered to a single rat. Two additional animals were dosed if no mortality was seen. They were monitored for the gross morphological changes and mortality up to 48 hours.

2.4 Experimental design:

Dose selection: Based on the literature survey the dose for the study was selected.
Based on acute oral toxicity study, 150mg/kg dose of BBS was selected for the study.

36 rats were divided into six groups and six animals in each group. The study period was conducted from 0–28 days in two different durations as 0–28 (developing phase) and 14–28 (developed phase) days. Indomethacin (3 mg/kg, po) was used as standard(Kumar, R., Gupta, Y. K., & Singh 2016). Both the standard and sample solutions were prepared in suspension in 1% sodium carboxy methyl cellulose (SCMC) were prepared freshly and used immediately.

Group I: Normal control (Receive standard feed and water *ad libitum).*

Group II: Arthritic control (Single dose of FCA, 0.1 ml/rat, sc injected at intraplantar region of left paw)

Group III: standard treatment in developing phase (Single dose of FCA, 0.1 ml/rat, sc injected at intraplantar region of left paw + received 3 mg/kg p.o. of indomethacin for 28 days).

Group IV: BBS treatment in developing phase (Single dose of FCA, 0.1 ml/rat, sc injected at intraplantar region of left paw + revived BBS 150 mg/kg, po for 28 days).

Group V: standard treatment in developed phase (Single dose of FCA, 0.1 ml/rat, sc injected at intraplantar region of left paw + received 3 mg/kg p.o) of indomethacin for 14 days from the 14th day to 28th day).

Group VI: BBS treatment in developed phase (Single dose of FCA, 0.1 ml/rat, sc injected at intraplantar region of left paw + revived BBS 150 mg/kg, po for 14 days from the 14th day to 28th day).

2.5 Induction of arthritis:

The rats were anesthetised with pentobarbitone sodium (40 mg/kg, ip) and injected with 0.1 ml of the Freund's complete adjuvant solution (FCA)at the intraplantar region of left hind paw. The right hind paw was kept as control.
As mentioned in the experimental groupings, the treatments were given. After the FCA injection, body weight, paw thickness and paw volume and paw thickness of all the experimental animals were measured on 0, 7, 14, 21 and 28th day. The mean change in paw volume and pw thickness with respect to initial paw volume and paw thickness was calculated on respective days and the percentage inhibition of paw edema was calculated by formula-

$$\% \text{ Inhibition} = \frac{\text{Control (Vc)-Test(Vt)}}{\text{Control(Vc)}} \times 100$$

The photographs of the rat hind limbs of all the experimental groups were taken. At the end of the experiment, the blood was collected through retro-orbital plexus from all the experimental rats. Then, they were euthanized with overdose of pentobarbitone sodium (150 mg/kg, if). The proximal interphalangeal joints from all the animals were isolated, fixed in 10% formalin solution for histopathological studies(Petchi et al. 2015)(Perumal, Ekambaram, and Dhanam 2017).

2.6 Analysis of hematological parameters:
The haematological parameters such as WBC counts and erythrocyte sedimentation were estimated manually using fresh blood of the experimental animals.

2.7 Analysis of biochemical parameters:
Serum samples were collected after centrifugation of whole blood at 3000 RPM for 20 min(Petchi et al. 2015). Liver markers such as alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT) and C-reactive protein (CRP) using standard kits in Robonik Pritest Bio autoanalyzer.

2.8 Statistical analysis:
The values are expressed as mean ± standard error of the mean. Statistical difference between normal to control and control to drug treatments were analyzed by One-way analysis of variance and Student's t-test was used to assess differences between multiple groups by using Graph Pad Prism software. The values of $P < 0.05$, 0.01, 0.001 and 0.0001 were considered statistically significant.

2. RESULTS:

3.1 Acute oral toxicity study:
BBS-containing formulation did not show any toxic or harmful effects up to 5000 mg/kg oral intake, indicating non-toxicity even at higher dose. The LD50 of BBS were found to be higher than 5000mg/kg. Hence, the dose of 150 mg/kg of BBS was chosen to assess in vivo antiarthritic activity in Freund's adjuvant produced arthritic rat model.

3.2 Effect of formulation containing Boswellic acid, Berberine and Silymarin on body weight of FCA induced arthritic rats.
Compared to normal group rats, FCA-induced arthritic did not show significant change in body weight. Indomethacin and formulation BBS treated group in developing and developed phase did not show significant change in body weight as compared to arthritic control. The results were shown in Table 1.

Table 1 Effect of formulation containing Boswellic acid, Berberine &Silymarin on body weight of FCA induced arthritic rats.

Animal group	Body weight (gm) in Mean ±SEM				
	0 Day	7th Day	14th Day	21st Day	28th Day
Group I	160.4±9.14	182±6.37	203.5±3.97	218.2±3.72	226±5.06
Group II	167.2±3.06^{ns}	179.2±4.55^{ns}	210.3±5.10 ^{ns}	218.7±6.11 ^{ns}	228.5±7.60 ^{ns}
Group III	161.2±2.66 ^{ns}	182.7±2.39 ^{ns}	208.8±2.50 ^{ns}	216.2±3.62 ^{ns}	225.8±4.66 ^{ns}
Group IV	161.3±2.38 ^{ns}	169.5±3.83 ^{ns}	197.7±4.46 ^{ns}	201±5.87 ^{ns}	234.2±6.00 ^{ns}
Group V	165.9±3.03 ^{ns}	176.7±3.18^{ns}	204.8±5.90 ^{ns}	212.2±6.90^{ns}	234.2±8.22 ^{ns}
Group VI	172.7±5.53^{ns}	183.7±6.56 ^{ns}	206.8±9.01 ^{ns}	225.3±9.77 ^{ns}	234.5±10.57 ^{ns}

Group I: normal control; group II: arthritic control; Group III: standard treatment in developing phase; Group IV: BBS treatment in developing phase; Group V: standard treatment in developed phase; Group VI: BBS treatment in developed phase;
Values are expressed as mean±SEM (n=6). *Represent statistical significance (*p<0.05, **P<0.01, ***P<0.001, ****p<0.0001)
a: is when compared with normal control group; b: is when compared with arthritic control group; ns is non-significant.

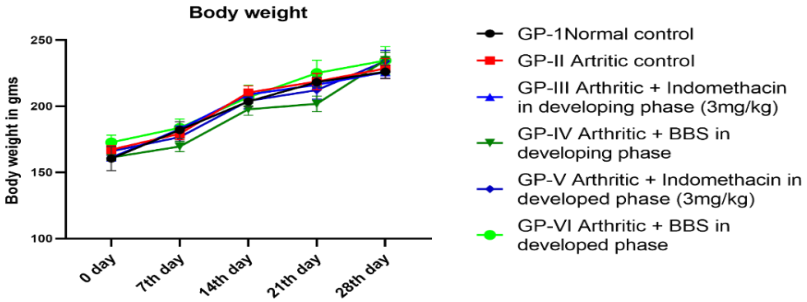

Figure 1Effect of formulation containing Boswellic acid, Berberine &Silymarin on body weight of FCA induced arthritic rats.

3.3 Effect of formulation containing Boswellic acid, Berberine and Silymarin on paw volume of FCA induced arthritic rats.
Compared to normal group rats, FCA-induced arthritic rats showed a significant ($p<0.0001$) increase in paw volume. In developing phase, standard (indomethacin) and formulation BBS treated group showed significant ($p <0.0001$ and $p<0.001$) reduction with percentage inhibition was 47.3 and 45.6% respectively when compared to arthritic control. In developed phase, standard (indomethacin) and formulation BBS treated group showed significant ($p <0.001$) reduction with percentage inhibition in paw volume was found to be 43.3 and 30.7 % respectively when compared to the arthritic control. The results were shown in Table 2 figure 2.

Table 2. Effect of formulation containing Boswellic acid, Berberine &Silymarin on paw volume of FCA induced arthritic rats.

Animal group	Paw volume (ml) in Mean ±SEM				
	0 Day	7th Day	14th Day	21st Day	28th Day
Group I	0.65±0.03	0.69±0.07	0.71±0.07	0.74±0.06	0.77±0.05
Group II	0.78±0.06^{ns}	1.11±0.03^{***a}	1.22±0.05^{***a}	1.39±0.02^{****a}	1.71±0.12^{****a}
Group III	0.71±0.0^{ns}	0.92±0.04^{*b} (17.3%)	0.97±0.06^{**b} (20.4%)	0.94±0.05^{****b} (32.3%)	0.9±0.04^{****b} (47.3%)
Group IV	0.65±0.04^{ns}	0.93±0.05^{*b} (12.6%)	0.99±0.08^{*b} (18.0%)	0.95±0.04^{***b} (24.4%)	0.93±0.02^{***b} (45.6%)
Group V	0.61±0.07^{ns}	0.96±0.05ns	1.13±0.06^{ns}	1±0.06^{***b} (28%)	0.97±0.02^{***b} (43.2%)
Group VI	0.73±0.03^{ns}	0.95±0.05ns	1.21±0.05^{ns}	1.10±0.03^{***b} (20.8%)	1.07±0.03^{***b} (30.7%)

Group I: normal control; group II: arthritic control; Group III: standard treatment in developing phase; Group IV: BBS treatment in developing phase; Group V: standard treatment in developed phase; Group VI: BBS treatment in developed phase;
Values are expressed as mean±SEM (n=6). * Represent statistical significance (*p<0.05, **P<0.01, ***P<0.001, ****p<0.0001). a: is when compared with normal control group; b: is when compared with arthritic control group; ns is non-significant.

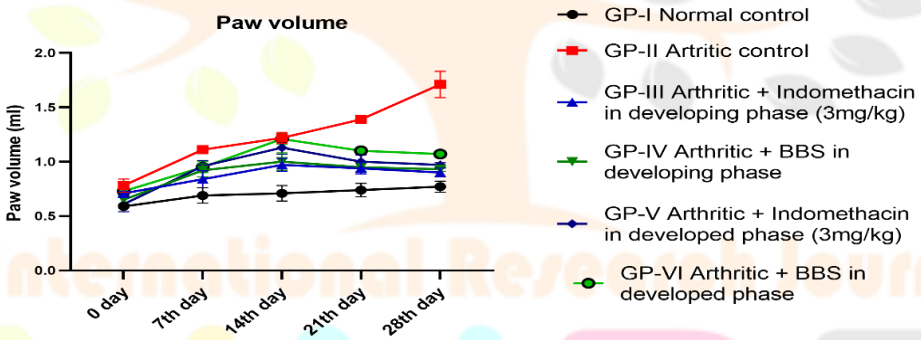

Figure 2Effect of formulation containing Boswellic acid, Berberine &Silymarin on paw volume of FCA induced arthritic rats.

3.4 Effect of formulation containing Boswellic acid, Berberine and Silymarin on paw thickness of FCA induced arthritic rats
Compared to normal group rats, FCA-induced arthritic rats showed a significant ($p<0.0001$) increase in paw volume.
In developing phase, standard (indomethacin) and formulation BBS treated group showed significant ($p <0.0001$ and $p<0.001$) reduction with percentage inhibition in paw thickness 29.04 %and 23.65 % respectively when compared to arthritic control. In developed phase, standard (indomethacin) and formulation BBS treated group showed significant ($p <0.0001$) reduction with percentage inhibition in paw volume was found to be 23.60% and 19.82 % respectively when compared to arthritic control. The results were shown in Table 3 And figure 3.

Table 3. Effect of formulation containing Boswellic acid, Berberine &Silymarin on paw thickness of FCA induced arthritic rats.

Animal group	Paw thickness (mm) in Mean ±SEM				
	0 Day	7th Day	14th Day	21st Day	28th Day
Group I	2.88±0.08	3.09±0.19	4.12±0.17	4.13±0.15	4.20±0.14
Group II	3.38±0.33^{ns}	4.20±0.38a* a	5.42±0.17^{***a}	5.59±0.14^{****a}	5.75±0.09^{****a}
Group III	2.8±0.14^{ns}	3.95±0.16^{ns} (5.95%)	5.05±0.18^{ns} (6.78%)	4.57±0.18^{**b} (18.24%)	4.08±0.16^{****b} (29.04%)

Group IV	3.00±0.20^{ns}	4.11±0.05^{ns} (2.38%)	5.26±0.11^{ns} (2.95%)	4.92±0.22^{*b} (11.9%)	4.39±0.20^{***b} (23.65%)
Group V	3.14±0.11^{ns}	4.17±0.12^{ns}	5.28±0.40^{ns}	5.04±0.19^{*b} (9.83%)	4.39±0.11^{****b} (23.6%)
Group VI	3.14±0.23^{ns}	4.17±0.04^{ns}	5.33±0.23^{ns}	4.96±0.18^{*b} (11.2%)	4.61±0.21^{***b} (19.82%)

Group I: normal control; group II: arthritic control; Group III: standard treatment in developing phase; Group IV: BBS treatment in developing phase; Group V: standard treatment in developed phase; Group VI: BBS treatment in developed phase;
Values are expressed as mean±SEM (n=6). * Represent statistical significance (*p<0.05, **P<0.01, ***P<0.001, ****p<0.0001). a: is when compared with normal control group; b: is when compared with arthritic control group; ns is non-significant.

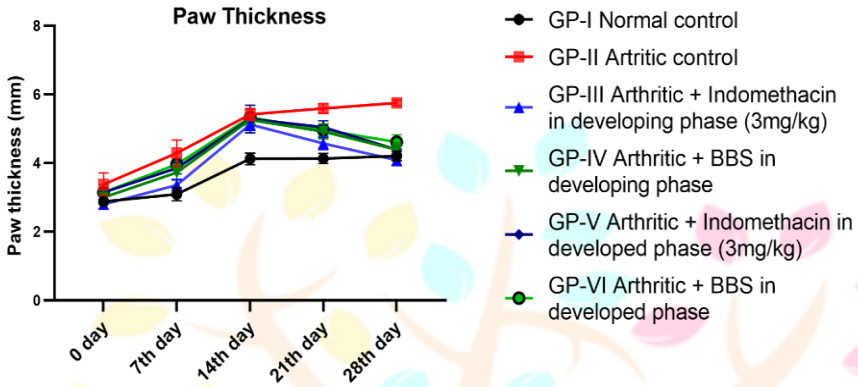

Figure 3Effect of formulation containing Boswellic acid, Berberine &Silymarin on paw thickness of FCA induced arthritic rats.

3.5 Effect of formulation containing Boswellic acid, Berberine and Silymarin on hematological parameter of FCA induced arthritic rats.

Compared to normal group rats, FCA-induced arthritic rats d showed significant (p<0.0001) increase in WBC count and ESR. In developing phase, standard (Indomethacin) and formulation (boswellic acid, berberine and silymarin) treated group showed significant (p<0.001 and p<0.001) reversal WBC count and ESR when compared to arthritic control group. In developed phase, standard (Indomethacin) and formulation (boswellic acid, berberine and silymarin) treated group showed significant (p<0.001 and p<0.01) reversal WBC count and ESR when compared to arthritic control group. BBS in developed phase showed moderate effect when compared to developing phase. The results were shown in Table 4.

Table 4. Effect of formulation containing Boswellic acid, Berberine &Silymarin on hematological parameters of FCA induced arthritic rats.

Animal groups	WBC in (Thousand cells/mm$^{3)}$	ESR (mm/hr)
Group I	7.57±0.32	0.33±0.21
Group II	12.22±0.28^{****a}	7.1±0.6^{***a}
Group III	8.7±0.34^{***b}	0.50±0.22^{***b}
Group IV	8.9±0.29^{***b}	0.67±0.21^{***b}
Group V	9.18±0.17^{***b}	1±0.36^{***b}
Group VI	9.57±0.28^{**b}	1.16±0.4^{***b}

WBC: white blood cell; ESR: erythrocyte sedimentation rate: Group I: normal control; group II: arthritic control; Group III: standard treatment in developing phase; Group IV: BBS treatment in developing phase; Group V: standard treatment in developed phase; Group VI: BBS treatment in developed phase; Values are expressed as mean±SEM (n=6). * Represent statistical significance (*p<0.05, **P<0.01, ***P<0.001, ****p<0.0001). a: is when compared with normal control group; b: is when compared with arthritic control group; ns is non-significant.

3.6 Effect of formulation containing Boswellic acid, Berberine and Silymarin on biochemical parameter of FCA induced arthritic rats.

Compared to normal group rats, FCA-induced arthritic control rats showed significant (p<0.0001) increase in ALP, AST, ALT and CRP level.

Indomethacin treated groups in developing and developed phase showed significant(P<0.001andp<0.05) reduction in elevated serum ALP, ALT, AST and CRP levels as compared to arthritic control rats. Whereas BBS in the developing phase of arthritis showed significant (P<0.001) reversal of ALP, AST, ALT and CRP level but BBS in developed phase showed moderate effect when compared to developing phase. The results were shown in Table 5.

Table 5. Effect of formulation containing Boswellic acid, Berberine &Silymarin on biochemical parameter of FCA induced arthritic rats.

Animal groups	ALP(U/L)	AST (U/L)	ALT(U/L)	CRP (mg/dl)
Group I	121.7±9.53	127.4±5.72	54.89±4.03	0.059±0.01
Group II	280.9±5.70^{****a}	221.8±8.73^{***a}	74.32±4.76^{****a}	0.308±0.02^{****a}
Group III	143.6±7.94^{****b}	140.1±7.59^{***b}	60.88±9.33^{****b}	0.159±0.01^{**b}
Group IV	181±.6±9.64^{****b}	131.29±3.84^{***b}	62.59±5.9^{****b}	0.162±0.01^{**b}
Group V	188.7±5.98^{****b}	184.6±8.56^{*b}	64.62±6.76^{*b}	0.206±0.01^{*b}
Group VI	211.3±8.37^{****b}	199.8±6.45^{ns}	63.71±7.37^{ns}	0.204±0.02^{*b}

ALP: Alkaline phosphatase; AST: Aspartate amino transferase; AST: Aspartate amino transferase; CRP: C-reactive protein.
Group I: normal control; group II: arthritic control; Group III: standard treatment in developing phase; Group IV: BBS treatment in developing phase; Group V: standard treatment in developed phase; Group VI: BBS treatment in developed phase;
Values are expressed as mean±SEM (n=6). * Represent statistical significance (*p<0.05, **P<0.01, ***P<0.001, ****p<0.0001).
a: is when compared with normal control group; b: is when compared with arthritic control group; ns is non-significant.

2.6 Histopathological study:

The histopathological results of inflamed hind limb of arthritic control were compared with that of the normal control group. normal control specimen showed intact joint space with intact synovial tissue and Synovial tissue is lined by intact synovial lining with underlying fibro connective tissue. Whereas arthritic control group showed significantly eroded synovial surface, cartilage and bone. Joint space is significantly narrowed. Synovial showed formation of pannus with dense fibro collagenous tissue deposit and severe infiltration of chronic inflammatory cells composed of lymphocytes, histiocytes and plasma cells along with numerous tiny blood vessels lined by endothelial cells. The group treated with indomethacin and BBS showed reduction in synovial cartilage and bone erosion, proliferation of collagenous tissue and increase in joint space. also showed reduced infiltration of chronic inflammatory cells composed lymphocytes, histiocytes and plasma cells (figure 1).

When compared to arthritic control group, BBS in developing phase showed significant reversal of all the above-mentioned pathological conditions but in developed phase moderate effect observed.

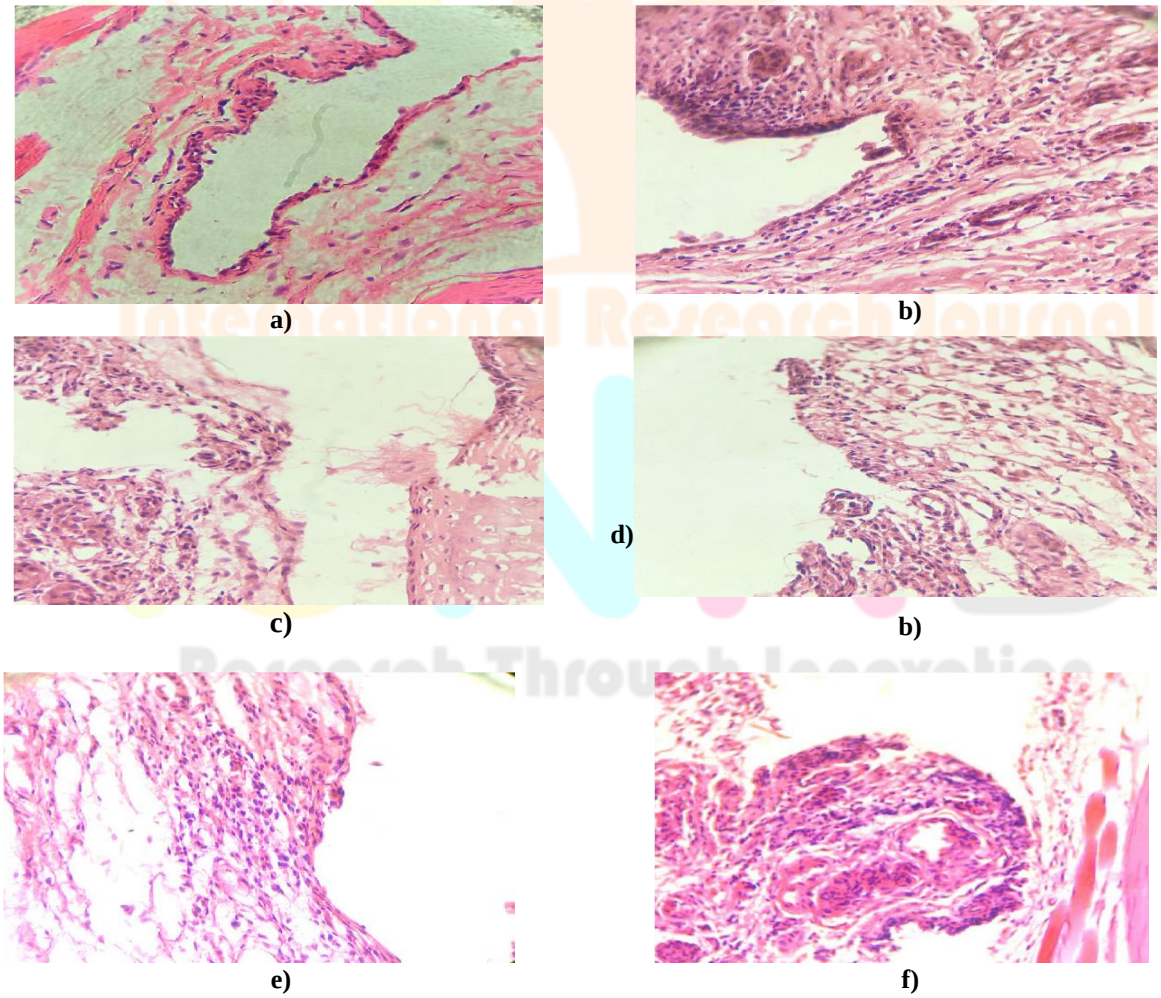

Figure 4. Histopathological results of interphalangeal joints on adjuvant-induced arthritic rats.
a) Group-I(normal control); b) Group-II(Arthritic control); c) Group-III (standard treatment in developing phase); d) Group IV(BBS treatment in developing phase); e) Group(standard treatment in developed phase); f) Group VI(BBS treatment in developed phase)

3. Discussion:

CFA-induced arthritis is one of the finest animal models of chronic polyarthritis, exhibiting characteristics similar to human RA and it involves cell mediated autoimmunity (Gohil et al. 2018). Therefore, Inhibition of CFA model is frequently used to evaluate antiarthritic drugs. The bacterial peptidoglycan and muramyl dipeptide found in the FCA are responsible for the induction of adjuvant arthritis(Petchi et al. 2015).

The anti-inflammatory effects on RA have been evaluated using changes in the rheumatoid indices, such as paw volume and paw thickness. (Saber et al. 2020)(Chen et al. 2017). From the results obtained, it was observed BBS and indomethacin treated groups showed continuous increase in paw volume and paw thickness from 0 to 14th day, after 14th day indomethacin (3mg/kg bw p.o) and BBS (at dose 150 mg/kg respectively) treated group in developing and developed phase showed significant decrease in the paw volume and paw thickness when compared to arthritic control group. The inhibition of BBS (in developing phase) obtained was nearly equal to that of the standard and was found to be effective. The antiarthritic effect of the BBS in the developing phase of arthritis showed better effect when compared to the developed phase of arthritis.

Change in the body weight is also a parameter to assess the course of the disease and the response to therapy. BBS treated group in developing phase and developed phase did not show significant change in the body weight which is almost similar to the weight of normal control group.

White blood cells are an important part of the body's immune system. Infections and inflammatory diseases are indications for a WBC count (Petchi et al. 2015). In the present study, arthritic control rats' WBC counts were found to be higher, which may be due to the stimulation of the immune system against the invading (Perumal, Ekambaram, and Dhanam 2017)(Chen et al. 2017).whereas the group treated with indomethacin and BBS showed significant decrease in WBC count in both developing and developed phase when compared to arthritic control group.

A frequent diagnostic sign of individuals with chronic arthritis is an increase in erythrocyte sedimentation rate, which was seen in the present investigation in the arthritic control group rats. The ESR is correlated with the number and size of RBCs as well as with the relative plasma protein concentration, particularly with fibrinogen and β globulins. An increase in the ESR is a sign of active but unidentified disease processes. The acute phase proteins in ESR cause inflammation comparable to that caused by an injection, injury, and surgery or tissue necrosis(Petchi et al. 2015). There was a decrease in ESR in groups treated with BBS in both developing phase and developed phase when compared to arthritic control group which indicating that significant recovery from the arthritic progress, thus establishing that formulation containing BBS has a significant role in arthritic conditions.

The assessment of the serum levels of ALP, ALT, AST and CRP is a tool to measure the antiarthritic activity of a particular drug. AST and ALT play an important role in the production of biologically active chemical mediators such as bradykinins in the inflammatory process. The elevated levels of ALP in the adjuvant-induced arthritic rats may be due to the increase in the liver and bone fraction. As a result, there is a localized loss of bone in the form of periarticular osteopenia and bone erosion. This is because the enzyme is released into circulation during the formation and resorption of bone(Borashan et al. 2008). CRP is a prototypic inflammatory biomarker of systemic inflammation that belongs to the class of acute phase proteins. The level of CRP raises during the inflammatory process that occurs in the body and the increase in the level of CRP is due to the rise in the plasma concentration of IL-6, which is generated by the macrophages and the adipocytes (Perumal, Ekambaram, and Dhanam 2017).

In the present study, the arthritic control rats in the control group showed elevated levels of ALP, ALT, AST and CRP when compared to normal control group. There was a significant decrease in ALP level, AST level, ALT level and CRP level in the group treated with BBS in developing phase when compared to arthritic control group. In developed phase of arthritis, BBS treated group showed moderate effect on ALP, AST, ALT and CRP level as compared to arthritic control group.

From the results of the present study, it can be stated the altered biochemical parameters were restored to near normal in BBS treated groups in both developing and developed phases of arthritis indicating that there may be reduction in bone loss and organ-protective mechanisms, possibly due to the decrease in the release of chemical mediators of inflammatory process.

Histopathological study shows the differences in the normal ankle joint and adjuvant-induced arthritic rat joint(Perumal, Ekambaram, and Dhanam 2017).In the present study arthritic control group showed significantly eroded synovial surface, cartilage and bone. Joint space is significantly narrowed. Synovial showed formation of pannus with dense fibro collagenous tissue deposit and severe infiltration of chronic inflammatory cells composed of lymphocytes, histiocytes and plasma cells along with numerous tiny blood vessels lined by endothelial cells. the group treated with indomethacin and BBS in developing phase showed marked reduction in all pathological above-mentioned conditions but in developed phase moderate effect is observed.

Thus, this study suggested that the antiarthritic effect of formulation containing BBS on joints, bone, and cartilage in FCA -induced arthritic rats was probably mediated by anti-inflammatory action. Therefore, BBS has significant potential as a phytomedicine and it is significantly effective in developing phase compared to developed phase.

4. Conclusion:

The current study has demonstrated that a formulation containing BBS (at doses of 150 mg/kg bw, po) has anti-inflammatory potential and exerts an anti-arthritic activity by significantly reversing the pathogenesis during FCA-induced arthritis in male albino wistar rats. The anti-arthritic potential of formulation containing BBS (150 mg/kg bw p.o) is comparable with that of indomethacin, additionally formulation of BBS is effective in developing phase compared to developed phase of Rheumatoid arthritis.

This study is limited to preclinical examination and the formulation of BBS is required chronic toxicity and pharmacokinetic studies to ensure the safety and efficacy of the formulation. further studies are needed to explore the underlying mechanism in molecular level.

5. Acknowledgement:

I am very grateful to the Principal, Prof. Dr. Shachindra L Nargund and Dr. L V Nargund, Founder of Nargund College Pharmacy, Bengaluru, for providing me the appropriate atmosphere for working hard for my research for their support and permission to undertake this study. I thank the VGST(GRD-178) and RGUHS for supporting the present work. The management at Nargund College of Pharmacy Bengaluru for supporting and providing the necessary infrastructural support. I would like to express our utmost and sincere gratitude to our guide Dr. Rama nargund, Nargund college of pharmacy, Bengaluru, extremely thankful to Dr. H.J Hrishikeshvan, HOD & Professor, Nargund college of pharmacy, Bengaluru.

6. Reference:

1. Almutairi, Khalid et al. 2020. The Global Prevalence of Rheumatoid Arthritis: A Meta-analysis Based on a Systematic Review. Rheumatology International: 863–877.
2. Borashan, Fatemeh Aghaei, Mino Ilkhanipoor, Mohammad Hashemi, and Farah Farrokhi. 2008. Investigation the Effects of Curcumin on Serum Hepatic Enzymes Activity in a Rheumatoid Arthritis Model. Electronic Journal of Biology, 4(4): 129–33.
3. Chen, Yong et al. 2017. Anti-Arthritic Activity of Ethanol Extract of Claoxylon Indicum on Freund's Complete Adjuvant-Induced Arthritis in Mice. BMC Complementary and Alternative Medicine, 17(1): 1–7.
4. Chunxia, Chen et al. 2011. Extracts of Arisaema Rhizomatum C.E.C. Fischer Attenuate Inflammatory Response on Collagen-Induced Arthritis in BALB/c Mice.Journal of Ethnopharmacology, 133(2): 573–82.
5. Cicero, Arrigo F G, and Alessandra Baggioni. 2016. Berberine and Its Role in Chronic Disease. Advances in Experimental Medicine and Biology, 928: 27–45.
6. Dragos, Dorin et al. Phytomedicine in Joint Disorders. *Nutrients.* 1(9): 1–18.
7. Gaba, Sobhi, Anjali Saini, Gurpreet Singh, and Vikramdeep Monga. 2021. An Insight into the Medicinal Attributes of Berberine Derivatives: A Review. Bioorganic and Medicinal Chemistry, 38(January): 116143.
8. Gohil, Priyanshee et al. 2018. Anti-Arthritic Activity of Cell Wall Content of Lactobacillus Plantarum in Freund's Adjuvant-Induced Arthritic Rats: Involvement of Cellular Inflammatory Mediators and Other Biomarkers. Inflammopharmacology, 26(1): 171–81.
9. Gomes, Raquel Pinheiro et al. 2013. Padronização de Modelo Experimental Adequado a Estudos Do Efeito Do Exercício Na Artrite. Einstein (São Paulo), 11(1): 76–82.
10. Gupta, O P et al. 1978. Anti-Inflammatory and Anti-Arthritic Activities of Silymarin Acting through Inhibition of 5-Lipoxygenase. Phytomedicine, 7(1): 21–24.
11. Iram, Farah, Shah Alam Khan, and Asif Husain. 2017. Phytochemistry and Potential Therapeutic Actions of Boswellic Acids: A Mini-Review. Asian Pacific Journal of Tropical Biomedicine, 7(6): 513–23. http://dx.doi.org/10.1016/j.apjtb.2017.05.001.
12. Kumar, R., Gupta, Y. K., & Singh, S. 2016. Anti Inflamatory Activity of Berberies Aristata.Pdf. in experimental models of inflammation. Indian journal of pharmacology, 48(2): 155–161.
13. Mahdi, Harith Jameel et al. 2018. 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 ,7(1): 85–94.
14. Perumal, Senthamil Selvan, Sanmuga Priya Ekambaram, and T Dhanam. 2017. In Vivo Antiarthritic Activity of the Ethanol Extracts of Stem Bark and Seeds of Calophyllum Inophyllum in Freund ' s Complete Adjuvant Induced Arthritis. Pharmaceutical Biology, 55(1): 1330–36.
15. Petchi, RRamesh et al. 2015. Antiarthritic Activity of a Polyherbal Formulation against Freund′s Complete Adjuvant Induced Arthritis in Female Wistar Rats.Journal of Basic and Clinical Pharmacy, 6(3): 77.
16. Roy, Nand Kishor et al. 2019. An Update on Pharmacological Potential of Boswellic Acids against Chronic Diseases. *International Journal of Molecular Sciences*, 20(17): 4101.
17. Saber, Marwa S et al. 2020. Assessment of the Preventive Effects of Silybum Marianum (L .) Gaertn . Seeds Hydroethanolic Extract and Silymarin on Complete Freund ' s Adjuvant-Induced Arthritis in Wistar Rats Assessment of the Preventive Effects of Silybum Marianum (L .) Gaertn . S. Annals of Phytomedicine: An International Journal, 9(2): 172-182.
18. Scherer, Hans Ulrich, Thomas Häupl, and Gerd R. Burmester. 2020. The Etiology of Rheumatoid Arthritis. Journal of Autoimmunity,110(December 2019): 102400.
19. Veronika, Mgr. 2020. MILK THISTLE (SILYBUM MARIANUM): A VALUABLE MEDICINAL PLANT WITH SEVERAL THERAPEUTIC PURPOSES. journal of microbiology, biotechnology and food science. 9: 6–8.
20. Yen-Ju Lin 1, Martina Anzaghe 2, and Stefan Schülke 1. Update on the Pathomechanism, Diagnosis,and Treatment Option for Rheumaoid Arthrit. Cells, 9(4): 880.