Pharmacological Evaluation of Anti-arthritic activity of *Vigna mungo* L. Seed Extract in Complete Freund's Adjuvant Induced Arthritis in Rats

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Abstract

Rheumatoid arthritis (RA) is a chronic, progressive autoimmune disorder characterized by synovial inflammation, cartilage destruction, and joint deformity, leading to pain and disability. Current pharmacological treatments, although effective, are associated with significant side effects, highlighting the need for safer alternatives. *Vigna mungo* (L.) Hepper, commonly known as black gram, has been traditionally used in Ayurveda and Siddha medicine for its anti-inflammatory, antioxidant, and immunomodulatory properties. The present study aimed to evaluate the anti-arthritic potential of the methanolic seed extract of *Vigna mungo* using the Complete Freund's Adjuvant (CFA)-induced arthritis model in rats. Arthritis was induced by sub-plantar injection of CFA (100µL) into the right hind paw of rats, and the animals were treated orally with *Vigna mungo* seeds extract at doses of 200 mg/kg and 400 mg/kg for 21 days. Methotrexate (1 mg/kg, *i.p.*) served as the standard drug. The efficacy of the extract was evaluated through changes in paw volume, arthritis score, body weight, hematological indices (RBC, WBC, Hb, ESR), biochemical and immunological parameters (CRP, RF, TNF-α, IL-6, IL-1β), and histopathological examination of joint tissues. The extract treatment significantly reduced paw edema and arthritis score in a dose-dependent manner, normalized hematological and biochemical parameters, and markedly decreased the levels of pro-inflammatory cytokines. Histopathological studies confirmed attenuation of synovial hyperplasia, pannus formation, and cartilage erosion. The findings suggest that *V. mungo* seed extract exerts potent anti-arthritic activity, likely mediated through suppression of inflammatory mediators and oxidative stress.

Keywords: Anti-arthritic activity, Complete Freund's adjuvant, Cytokines, *Vigna mungo* L., Rheumatoid arthritis

Introduction

Arthritis, a broad term encompassing more than 100 different types of joint diseases, is a debilitating condition that affects millions of people globally. Among its variants, rheumatoid arthritis (RA) is a progressive, systemic autoimmune disorder characterized by chronic inflammation of synovial joints, leading to cartilage degradation, bone erosion, and joint deformity. The global burden of RA is significant, affecting approximately 0.5–1% of the adult population, with a higher prevalence in women than in men [1, 2].

Current therapeutic regimens, including non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, disease-modifying anti-rheumatic drugs (DMARDs), and biological agents, are effective in managing symptoms and slowing disease progression [3]. While these therapies can alleviate symptoms and slow disease progression, they often come with substantial side effects and are not curative, however, their long-term use is often limited by adverse effects such as gastrointestinal toxicity, immunosuppression, hepatotoxicity, and high costs, especially in resource-limited settings [4]. This has led to increased interest in exploring alternative therapeutic options, particularly from natural sources, that offer efficacy with minimal side effects. Numerous plants used in traditional medicine have shown potential anti-inflammatory, immunomodulatory, and antioxidant properties relevant to arthritis treatment. One such plant is *Vigna mungo* L., commonly known as black which belongs to the family Fabaceae.

Vigna mungo L., commonly known as black gram, is a leguminous plant widely cultivated in tropical and subtropical regions, especially in India, where it is a staple pulse crop. Apart from its nutritional value, *Vigna mungo* has a rich history in traditional Ayurvedic medicine. Traditionally used in Ayurvedic and Siddha medicine, *Vigna mungo* seeds have been reported to possess tonic, diuretic, aphrodisiac, anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory properties [5-7]. The presence of bioactive compounds such as flavonoids, phenolic acids, tannins, and alkaloids might be playing a key role in modulating inflammatory pathways. However, scientific evaluation of its potential in the management of chronic

inflammatory conditions like rheumatoid arthritis remains limited and underexplored. Given its traditional use and pharmacological profile, the seed extract of *Vigna mungo* could be a valuable candidate for anti-arthritic drug development. Hence, the present study is designed to evaluate the anti-arthritic activity of *Vigna mungo* seed extract using the Complete Freund's Adjuvant (CFA) induced arthritis in rats.

Material and Methods

Collection and authentication of *Vigna mungo* Seed

The *Vinga mungo* seeds were collected from local market in and around of Coimbatore. The seed were botanically identified and authenticated by The Joint Director, Botanical Survey of India, Coimbatore, Tamil Nadu, India, and the specimens were stored in the department of Pharmacology, Sri Abirami College of Pharmacy, Coimbatore for further references (Specimen Ref. no.: 002/2025).

Preparation and Extraction of *Vigna mungo* Seed

The raw whole dried seed of *Vinga mungo* were coarsely powdered and sieved (mesh no. 40). The 100 gm of powdered materials were defatted with petroleum ether (40-60°C) and then extracted with 50% v/v ethanol by cold maceration technique for 48 hrs. After completion of the extraction, it was filtered and dried at 60°C in rotary evaporator to produce a semisolid mass. The dried extract was stored at 4°C in an air tight container until further use.

Preliminary phytochemical analysis

The ethanolic seed of *Vinga mungo* (ESVM) was subjected to preliminary phytochemical analysis for identifying the phytoconstituents Carbohydrates, flavonoids, phenols, alkaloids, tannins, saponins, terpenes, steroids, and glycosides present in the extract. The tests were carried out by standard procedures [8]**.**

Acute oral toxicity studies

Acute oral toxicity of *V. mungo* seeds ethanolic extract was evaluated by the acute toxic class method as per OECD (Organization for Economic Co-operation and Development) test guideline 423 [9]. Three female rats were treated with a single oral dose of ESVM 2000 mg/kg. After administration, each animal was individually observed for the first 30 minutes, followed by special attention for the first 4 hours and periodically for 24 hours, thereafter daily for 14 days. The evaluation parameters include changes in skin and fur, mucous membrane, eye, respiration, circulatory, central, peripheral nervous system, and somatomotor activity and behavioral

changes. The occurrence of salivation, diarrhea, lethargy, tremors, convulsions, sleep, and coma should be monitored closely.

***In vivo* anti-arthritic studies**

Selection of animals

The colony inbred mature Albino wistar rats (180-220 g) were included in this study. The animals were kept under standard environmental conditions of 12/12 light/dark rhythm, maintained under controlled (23 ±2°C) room temperature. They were fed with standard pellet diet and water *ad libitum*. The immature animals were acclimatized under laboratory conditions 7 days prior to initiation of experiment. The cages were cleaned daily by changing the sawdust bedding. The Experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Sri Abirami College of Pharmacy, Coimbatore; Care and use of laboratory animals were confirmed to CCSEA guidelines (IAEC Reference No: SACOP/Re/M. Pharm/07/2025 Dated: 21.06.2025).

Induction of CFA induced Arthritis

Adjuvant arthritis was induced by single subcutaneous injection of Complete Freund's adjuvant (CFA) (100µl of 5mg/ml suspension of Heat killed *Mycobacterium tuberculosis* organism in liquid paraffin) in to sub planter tissue of the right hind paw of each rat except vehicle treatment group using 26 gauge needle. Paw volume was measured after six hour of CFA injections was considered as day1 measurement. The drug treatments were started after induction of arthritis on day 9 and continued till 28th day of study [10].

Experimental Design

Thirty male albino Wistar rats were (180 -220 gm) were included and randomly divided into five groups of six per each, Group I served as normal control received normal saline (0.5 ml), *p.o*., Group II-V treated with CFA (100µl in to sub plantar region) to induced arthritis, Group II served as arthritic control receives 0.5 ml of 0.5% CMC *p.o.* Group III-V served as treatment group received Methotrexate (1 mg/kg once a week *i.p.*), *V. mungo* seed extract 200 mg/ kg/day and 400 mg/ kg/day *p.o.* for 21 days respectively.

Evaluation of Arthritis

Bodyweight changes

Body weight was measured before inducing arthritis, and weekly until day 28 of the study via digital weighing balance.

Measurement of Paw volume

The primary and secondary responses i.e. paw volume of ipselateral and contra lateral hind paw of each rat was measured before induction and further determined on day 7, 14, 21 and 28 by using liquid displacement plethysmometer.

Assessment of arthritis severity index

The severity of arthritis was recorded by a blinded observer using the visual arthritis scoring system. The arthritis score ranged from 0 to 4; 0 indicates the least but definite swelling and 4 represent the maximum swelling. This scoring systems involves observations of all four paws and giving a separate score for each limb. Scores are assigned for evaluation of the pain associated with the arthritis as shown in Table 1[11].

Table 1: Arthritis Severity Index

Score	Flexion pain test score	Mobility score	Stance score
0	No squeaking and no leg withdrawal	Normal	_
1	Either squeaking or leg withdrawal	Limping	Paw lift continuously
2	Both squeaking or leg withdrawal	Walking with difficulty	Paw touching but with no weight bearing
3	_	Walking without touching the injected paw	Some weight bearing on the paw
4	_	_	Normal

Evaluation of Arthritis from Blood Parameters

On the 29th day, blood samples were collected by retro orbital plexus from diethyl ether anaesthetized rats into plain and ethylenediamine tetraacetic acid (EDTA) tubes and subjected to hematologic and biochemical testing parameters through the chemical analyzer by using commercially available kits.

Haematological analysis

The hematological parameters like RBC, WBC, Hb, PCV, and ESR were analyzed in the blood sample collected in the EDTA tube using hematological auto analyzer (Mindray BC-700).

Serum Biochemical analysis

Blood samples collected in the non-heparinized tubes were allowed to clot. Then the serum was separated from the clotted blood by centrifuge at 2,000 x g for 10 minutes, and then the supernatant fluid of serum was transfer into clean eppendrof tube for the biochemical analysis of Rheumatoid Factor (RF), Serum C - reactive protein (CRP), Serum TNF-α, IL-6, and IL-1β were assessed by ELISA technique using standard marketed kits.

X-ray analysis of the ankle joint and paws

On 29th day the ankle joint and paws of each rat are examined by X-ray analysis under anesthesia. X-ray was taken at the joint of the contralateral paw for the confirmation of the severity of arthritis in CFA induced rats [11].

Immune Organ Weight measurement

All the rats were sacrificed on 29th day of the study following over dose anesthesia. The spleen and thymus were removed from each rat, clean and weighed by using standard weighing balance.

Histopathological analysis of Joint tissues

The right hind limb was removed from the rats for histopathological examination. The ankle joints were detached, wash with distilled water and retained in 10% formalin then decalcified by putting them in decalcifying solution for 1 month. Joints were then trimmed, embedded in paraffin wax and sectioned at 6 µm. The tissue sections were stained with hematoxylin and eosin (H&E). The ankle joints were observed for inflammation, bone erosion and pannus formation under microscope [11].

Statistical analysis

The data represents a Mean ± SEM (Standard Error Mean) of six replicated determinations. Results were analyzed statistically by one way ANOVA followed by followed by Tukey's multiple comparisons test for normal data and by the Kruskal-Wallis test for the scored data using GraphPad Prism-8.0.2 version. The difference was considered significant when $p<0.05$.

Results

Extraction Value and Preliminary Phytochemical analysis

The cumulative extraction value of the *Vinga mungo* seed ethanolic extract was calculated as 12.4% w/w yield percentage. The primary phytochemical screening of ethanolic seeds extract of

V. mungo (ESVM) showed the presence of alkaloids, carbohydrates, steroids, proteins, tannins, phenols, steroiols & terpens.

Acute toxicity study

The acute oral toxicity of ethanolic seed extract of *V. mungo* at the limited dose 2000 mg/kg single dosing revealed that normal weight gain in the treated animals. There were also no toxic symptoms, mortality, observational, behavioral and somatomotor changes observed after single dosing of ESVM. These results indicate that the ESVM was found to safer for acute use at the tested dose level of 2000 mg/kg. Hence the lethal dose of 50% (LD50) of *V. mungo* is greater than 2000 mg/kg. Based on the acute oral toxicity results considering the body surface factor $1/10^{th}$ of the maximum 2000 mg/kg acute oral toxicity dose of 200 mg/kg was selected as the low dose, twofold higher than the low dose 400 mg/kg was selected as the high dose.

Effect of ethanolic seed extract of *V. mungo* against CFA induced paw oedema on ipselateral paw (Primary lesions)

The possible anti-inflammatory effect of ethanolic seed extract of *V. mungo* on rheumatoid arthritic rats was evaluated by measuring the paw volume of the ipselateral paw. After onset of inflammation the peak incidence in swelling is reached during 7^{th} - 9^{th} day with the increase in paw volume at the maximum of 0.64 ml for all the groups. The 21 days treatment of ESVM showed dose dependent significant ($P<0.001$) inhibition of paw volume was observed than the normal control. All the treatments showed equal effect on reduction in paw volume (Table 2).

Table 2: Effect of ESVM against complete Freund's adjuvant induced paw oedema on ipselateral paw (Primary lesions)

Groups	Mean Paw Volume of ipselateral paw (in ml)					
	Day 0	Day 1	Day 7	Day 14	Day 21	Day 28
Group-I (Vehicle Control)	0.42±0.01	0.42±0.03	0.43±0.02	0.43±0.02	0.44±0.02	0.44±0.03
Group-II (Arthritic control)	0.43±0.02	0.68±0.02 a***	0.58±0.01 a*	0.64±0.04 a**	0.72±0.05 a***	0.78±0.03 a***
Group-III	0.42±0.03	0.70±0.03	0.59±0.03	0.60±0.01	0.54±0.02	0.52±0.02

(Methotrexate 1 mg/kg)		a***	a*	a*	b***	b***
Group-IV (ESVM-200 mg/kg)	0.42±0.02	0.67±0.03 a***	0.60±0.02 a*	0.60±0.02 a*	0.56±0.02 b***	0.53±0.03 b***
Group-V (ESVM-400 mg/kg)	0.43±0.02	0.68±0.02 a***	0.59±0.02 a*	0.58±0.02 a*	0.53±0.03 b***	0.50±0.01 b***

Values are expressed as mean ± SEM, n=6. Comparisons were made between: a – Group I vs II, III, IV and V. b – Group II vs III, IV and V. Symbols represent statistical significance: ***- p< 0.001, ** - p< 0.01, * - p< 0.05

Effect of ESVM against CFA induced paw oedema on contra lateral paw (secondary lesions)

The secondary lesions that occurs after 21 days of CFA injection, characterized by joint swelling and nodule formation in the contra lateral paw and lymph node was first evident on 14th day and maximum swelling was found on day 21th. All the treatments showed significant (p<0.001) reduction in paw volume when compare to the arthritic control. Hence the treatment of ESVM possesses significant and earlier protection against joint swelling and nodule formation and prevents the disease progression (Table 3).

Table 3: Effect of ESVM against CFA induced paw oedema on contra lateral paw (secondary lesions)

Groups	**Mean Paw Volume of contra lateral paw (in ml)**					
	Day 0	**Day 1**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Group-I (Vehicle Control)	0.42±0.03	0.43±0.03	0.43±0.02	0.44±0.01	0.44±0.02	0.45±0.04
Group-II (Arthritic control)	0.42±0.02	0.42±0.03	0.44±0.01	0.56±0.04	0.62±0.05 a***	0.66±0.04 a***
Group-III (Methotrexate 1	0.43±0.02	0.43±0.02	0.45±0.03	0.48±0.02	0.50±0.02 b***	0.50±0.02 b***

mg/kg)						
Group-IV (ESVM-200 mg/kg)	0.43±0.01	0.43±0.02	0.46±0.02	0.47±0.01	0.56±0.02 b**	0.52±0.02 b***
Group-V (ESVM-400 mg/kg)	0.42±0.02	0.42±0.02	0.45±0.02	0.46±0.03	0.47±0.03 b***	0.45±0.02 b***

Values are expressed as mean ± SEM, n=6. Comparisons were made between: a – Group I vs II, III, IV and V. b – Group II vs III, IV and V. Symbols represent statistical significance: ***- p< 0.001, ** - p< 0.01, * - p< 0.05

Effect of ESVM on arthritic pain index

The treatment of ESVM altered the pain scores and arthritic score, indicating a significant decrease in the pain associated with the adjuvant-induced arthritis (Table 4**).** All the evaluated pain scores, including the flexion pain test score, mobility score and stance score were dose dependently altered in the *V. mungo* seed extract treated rats. The reduction in the mobility score, flexion pain test score, arthritic score and increase in stance score was greater in the high dose of ESVM (400 mg/kg) treated group as compared with the other treatment groups.

Table 4: Effect of ethanolic seed extract of *V. mungo* on arthritic pain index

Group	Arthritis score	Flexion pain test score	Mobility score	Stance score
Group-II (Arthritic control)	12 (9, 14)	2 (1, 2)	3 (2, 3)	1.5 (1, 2)
Group-III (Methotrexate 1 mg/kg)	6 (4, 8) **	2 (1, 3)	2.5 (1, 3)	3 (2, 5)
Group-IV (ESVM-200 mg/kg)	4 (3, 6) ***	1.5 (1, 2) *	1 (0, 1) **	4.2 (2, 5) **
Group-V (ESVM-400 mg/kg)	3 (2, 5) ***	1.25 (0, 2)	1.5 (1, 2) *	3.1 (2, 4) *

Values were represented in median (minimum, maximum), n=6. Symbols represent statistical significance: ***- p< 0.001, ** - p< 0.01, * - p< 0.05

Effect of ESVM in Changes on body weight

The average gain in the body weight on day 28 as compared with the initial body weight in each treatment group has been given in Table 5. A significant ($p < 0.001$) increase in body weight was observed in *CFA* arthritic control when compared to the control group. The changes in body weight gain were significantly less in the treatment groups when compared to the arthritic control group.

Table 5: Effect of ESVM in Changes on body weight

Groups	Initial body weight (g)	Final body weight (g)	Changes in body weight (g)
Group-I (Vehicle Control)	188.25 ± 14.04	213.25 ± 7.69	25.00 ± 1.63
Group-II (Arthritic control)	185.50 ± 9.23	235.50 ± 5.25 ^{a**}	50.00 ± 1.16^{a***}
Group-III (Methotrexate 1 mg/kg)	187.50 ± 7.81	219.25 ± 4.57	31.75 ± 2.45^{a*}
Group-IV (ESVM-200 mg/kg)	186.25 ± 8.43	215.00 ± 5.48	28.75 ± 0.71^{b**}
Group-V (ESVM-400 mg/kg)	186.00 ± 10.08	218.00 ± 6.29	32.00 ± 0.41^{a*}

Values are expressed as mean ± SEM, n=6. Comparisons were made between: a – Group I vs II, III, IV and V. b – Group II vs III, IV and V. Symbols represent statistical significance: ***- $p < 0.001$, ** - $p < 0.01$, * - $p < 0.05$

Effect of ESVM on Haematological parameters in CFA induced Arthritic rats

The results in table 6 represent the effect of ESVM on hematological parameters in CFA induced arthritic rats. Level of RBC, Hb, and PCV were decreased significantly ($P<0.001$) in arthritic rats with concomitant increases ($P<0.001$) in WBC and ESR levels. These changes were significantly reverted in all treatment groups. The treatment of ESVM showed dose dependent effect beneficial effect on hematological parameters in the arthritic rats (Figure 14).

Table 6: Effect of ESVM on Haematological parameters in CFA induced Arthritic rats

Groups	RBC ($x10^6$/µl)	WBC ($x10^3$/µl)	Hb (g/dl)	ESR (mm/1st hr)	PCV (%)
Group-I (Vehicle Control)	6.98±0.23	8.42±0.51	14.50±1.29	3.2±0.10	53.23±1.67
Group-II (Arthritic control)	4.20±0.11 a***	13.65±0.27 a***	10.30±0.32 a***	11.6±0.78 a***	28.90±4.76 a***
Group-III (Methotrexate 1 mg/kg)	5.00±0.09 a***	7.89±0.61 b***	12.67±0.10 a* b**	5.4±0.44 a** b***	40.41±8.00 b***
Group-IV (ESVM-200 mg/kg)	7.01±0.27 b***	9.40±0.63 b***	13.46±0.40 b***	4.3±0.21 b***	46.99±1.01 b***
Group-V (ESVM-400 mg/kg)	7.20±0.67 b***	8.89±0.43 b***	14.68±1.51 b***	3.7±0.29 b***	49.21±2.31 b***

Values are expressed as mean ± SEM, n=6. Comparisons were made between: a – Group I vs II, III, IV and V. b – Group II vs III, IV and V. Symbols represent statistical significance: ***- p< 0.001, ** - p< 0.01, * - p< 0.05

Effect of ESVM on serum inflammatory markers in CFA induced Arthritic rats

The arthritic control group showed a significant (P<0.001) increase in the serum levels of RF, CRP, TNF-α, IL-6 and IL-1β, whereas the methotrexate and ESVM low dose and high dose treatment groups showed significant (P<0.001) decrease level of serum RF, CRP, TNF-α, IL-6 and IL-1β in arthritic rats. The ESVM showed dose dependent beneficial effect on the serum inflammatory markers in arthritic rats (Table 7).

Table 7: Effect of ESVM on serum inflammatory markers in CFA induced Arthritic rats

Groups	RF (IU/ml)	CRP (mg/dl)	TNF-α (Pg/ml)	IL-6 (Pg/ml)	IL-1β (Pg/ml)

Group-I (Vehicle Control)	8.2±0.45	2.0±0.25	14.4±2.87	85.12±9.00	34.5±1.33
Group-II (Arthritic control)	52.3±0.20 a***	8.9±0.45 a***	49.5±5.28 a***	140.20±6.43 a***	89.4±8.30 a***
Group-III (Methotrexate 1 mg/kg)	23.6±0.91 a*** b***	3.7±0.81 b***	24.3±1.10 b***	99.23±3.55 b***	45.0±1.98 b***
Group-IV (ESVM-200 mg/kg)	32.3±0.07 a*** b***	4.2±0.10 b***	29.0±4.32 a** b***	82.12±5.20 b***	55.2±2.35 a** b***
Group-V (ESVM-400 mg/kg)	12.0±0.23 a** b***	2.9±0.22 b***	17.7±3.45 b***	84.50±6.49 b***	48.2±4.31 b***

Values are expressed as mean ± SEM, n=6. Comparisons were made between: a – Group I vs II, III, IV and V. b – Group II vs III, IV and V. Symbols represent statistical significance: ***- p< 0.001, ** - p< 0.01, * - p< 0.05

Effect of ESVM on gross lesions on X-ray analysis of the ankle joint and paws in CFA induced Arthritic rats

Figure 1 shows the X-ray analysis of rat paws, no evidence of pathological changes was observed in vehicle control group animal. The arthritic control rat paw shows severe inflammation with diffused joint space and bone erosion. The methotrexate and the ESVM low dose and high dose treatment shows clear joint space with no evidence of bone erosion and inflammation.

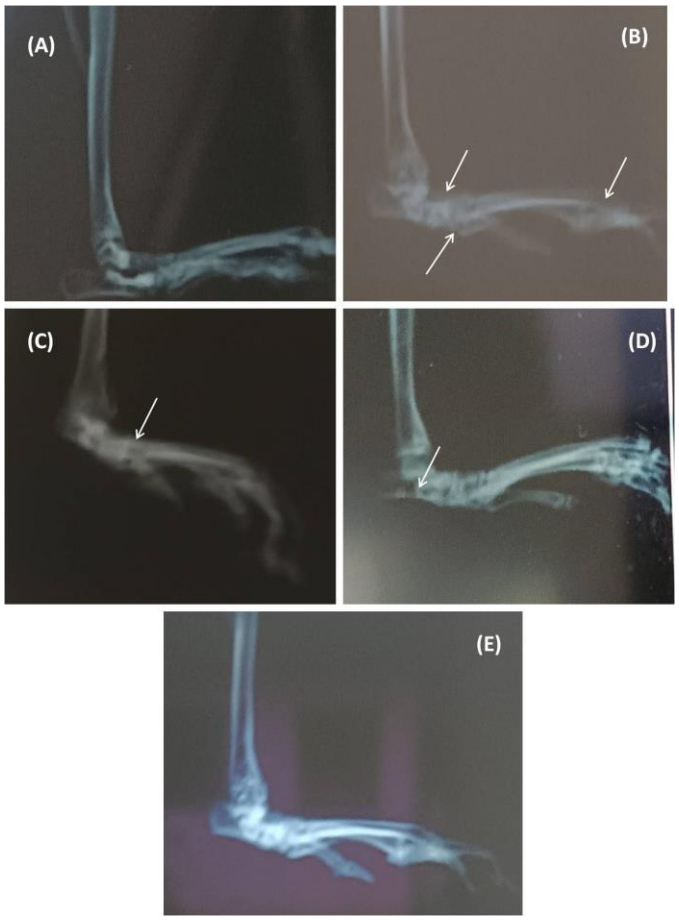

Figure 1: Effect of ESVM on X-ray analysis of the ankle joint and paws in the rats on day 28. (A) Vehicle Control F; (B) Arthritic control; (C) Standard group- Methotrexate 1 mg/kg; (D) ESVM-200 mg/kg; (E) ESVM-400 mg/kg

Effect of ESVM on Changes in Spleen and Thymus weight in CFA induced Arthritic rats

The weight of thymus and spleen was significantly ($p<0.001$) increased in the arthritic control rats as compared to the vehicle control. The methotrexate and the ESVM low dose and high dose treatment showed significant decreases in thymus and spleen weight as compared to the arthritic control (Table 8).

Table 8: Effect of ESVM on Changes in Spleen and Thymus weight in CFA induced Arthritic rats

Group	Spleen weight (g/100g body weight)	Thymus weight (g/100 body weight)
Group-I (Vehicle Control)	0.26 ± 0.01	0.08 ± 0.01

Group-II (Arthritic control)	0.84 ± 0.01 a***	0.13 ± 0.01 a**
Group-III (Methotrexate 1 mg/kg)	0.48 ± 0.02 a*** b**	0.09 ± 0.01 b*
Group-IV (ESVM-200 mg/kg)	0.31 ± 0.01 b***	0.08 ± 0.01 b**
Group-V (ESVM-400 mg/kg)	0.28 ± 0.01 a** b***	0.08 ± 0.01 b*

Values are expressed as mean ± SEM, n=6. Comparisons were made between: a – Group I vs II, III, IV and V. b – Group II vs III, IV and V. Symbols represent statistical significance: ***- p< 0.001, ** - p< 0.01, * - p< 0.05

Effect of ESVM on Histopathological changes in joints of CFA induced Arthritic rats

The results in Figure 2 shows histopathological changes in joints of CFA induced Arthritic rats. Arthritic control showing severe joint cartilage destruction and high inflammatory cellular infiltrations. The treatments of ESVM showing dose dependent effect of normal joint architecture and no inflammatory cellular infiltration.

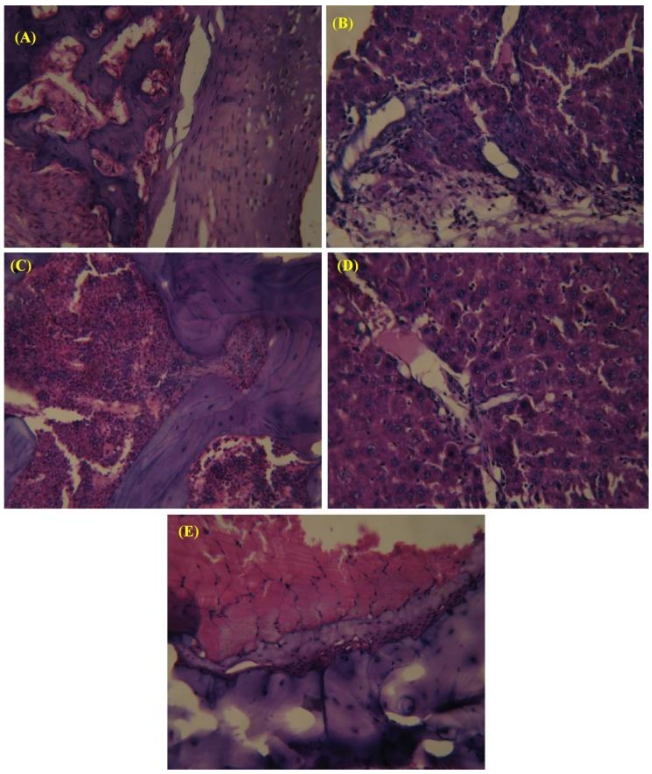

Figure 2: Effect of ESVM on Histopathological changes in joints of CFA induced Arthritic rats

Discussion

Rheumatoid arthritis (RA) is a complex, chronic, immune-mediated disease characterized by persistent synovial inflammation, joint destruction, bone erosion, systemic symptoms, and raised levels of pro-inflammatory cytokines (e.g. TNF-α, IL-1β, IL-6). Effective therapies exist (NSAIDs, corticosteroids, DMARDs, biologics), but their long-term use is limited by toxicity, cost, and sometimes partial efficacy. Hence there is substantial interest in botanicals and phytochemicals that may have anti-inflammatory, immunomodulatory, and antioxidant effects with fewer side effects.

Vigna mungo (black gram, *Māsh*) is a widely used legume in traditional Indian medicine, known to contain flavonoids, isoflavonoids, phenolic acids, saponins, alkaloids, etc. [12] Prior studies have reported its anti-inflammatory and analgesic properties (e.g. in carrageenan-induced paw edema) and immunostimulatory effects (on humoral and cellular responses) [13]. These findings make it plausible that seed extract could ameliorate rheumatoid arthritis in CFA model.

The acute oral toxicity study of ESVM did not show any toxic effects or lethality at the limit dose of 2000 mg/kg *p.o.*, which showed that the ethanoilc seed extract of *V. mungo* is safe to use up to 2000 mg/kg. According to the Globally Harmonized System (GHS) toxicity classification the tested ESVM was classified as category 5 or unclassified (2000 mg/kg < LD_{50} <5000 mg/kg).

After *CFA* injection on the right hind paw of the initial reaction (Primary lesions) of edema and soft tissue thickening developed at the depot site by the irritant effect of the adjuvant, whereas the last phase arthritis and flare in the injected foot is presumed to immunological events [14]. The occurrence of secondary lesion (swelling in the non-injected paw) characterized by tibiotarsal joint swelling and nodules formation in the tail is a manifestation of cell mediated immunity. Chronic inflammation involves the release of number of inflammatory mediators like pro-inflammatory cytokines (IL-1β and TNF-α), interferons, GM-CSF (Granulocytes-macrophage colony stimulating factor) and PGDF. These types of mediators are responsible for the pain (hyperalgesia), destruction of bone and cartilage can lead to severe disability [15]. It appears from our findings methotrexate and ethanolic seeds extract of *V. mungo* treatment group were significantly ($p<0.001$) reduced the primary and secondary lesions. When comparison were made between these treatments ESVM treated group possess equal and visually more action in

reducing the inflammation. It may be due to the inhibiting the release of inflammatory cells. ESVM treatment groups on day 21 of treatment significantly ($p<0.001$) reduced both the paw volume as compare to the arthritic control.

Arthritis index in the visual arthritis scoring system were assigned for evaluating the pain associated with arthritis and functional impairment in arthritis. In this study the arthritic score was significantly decreased in the entire treatment group as compared to arthritic control, it reveals the significant reduction of arthritis in the ESVM treated groups. Furthermore the ESVM-400 mg/kg treated group lowered the mobility score and improved the stance score indicating the reduction in pain and functional impairments in arthritic rats.

In arthritic condition moderate decreases in RBC count and Hb represent the anemic symptom in rats [16]. Hypochronic and normocytic anemia is generally seen in arthritic condition and it is due to reduction in the RBC count with a modest reduction in the PCV [17]. Reduction in the Hb during arthritis is due to the reduced erythropoietin level, a decreased response of the bone marrow erythropoietin and premature destruction of red blood cell. In this study all the treatment groups showed significant increase in RBC count, Hb, and PCV.

ESR is an indirect measurement of acute phase response for determining the RA disease activity [18]. The ESR attributed to the accelerated formation of endogenous proteins such as fibrinogen and α/β globulin, the increased level of ESR indicating the chronicity and severity of the disease activity. The increased ESR level in the arthritic condition was brought back by the treatment groups.

In arthritic condition there will be mild to moderate rise in WBC count due to release of IL-6, IL-1β inflammatory response. IL-1β increase the production of both granulocytes and macrophages colony stimulating factor [19] and also Lymphocyte count also elevated in arthritic condition, because T-lymphocytes have been reported to play a central role in the pathogenesis of RA [16]. In this study reveals that all the treatment group significantly revert the elevated WBC and Lymphocyte count. The above mentioned changes were more significantly ($p< 0.001$) brought back to near normal level upon the ESVM treatments. Which emphasizes the beneficial effect of the *V. mungo* seeds extract on CFA induced arthritic rats.

The serum C-reactive protein (CRP), rheumatoid factor (RF) and TNF-α, are markers of systemic inflammation and antibody production against the injected CFA. C- reactive protein is a member of the acute phase reactant, its level raised dramatically during inflammation [20]. Serum RF is the immunological expression of an individual immune system reaction to the presence of an immunoglobulin that is recognized as non self. Increased serum level of RF is due to the higher development of inflammation [21]. In this study the treatment groups showed significant reduction in serum CRP, RF and TNF-α. In comparison between these treatment groups, ESVM treated group shows more significant action on this biomarker of inflammation and autoimmune stimulation in CFA induced arthritic rats.

The reduction in spleen and increase in thymus weight are related to a stimulatory effect on immune system [22]. The observed decrease in the spleen and thymus weight in all the treatment groups indicates alteration of cell population in these organs, which are related to the immune function. It can be attributed the anti-proliferative action of ESVM. When comparison between these treatments group ESVM-400 mg/kg treated group have more antiproliferative action in these organs.

Radiographic changes in CFA induced rheumatoid arthritic rats are the diagnostic measures which indicate the severity of the disease. Soft tissue swelling around the ankle joint of arthritic rats was considered to be due to edema of periarticular tissues such as ligament and capsule [23]. In CFA induced RA rats, bone erosion representing the bony destruction were evident on bone unprotected by cartilage. Since this joint are exposed directly to proinflammatory cytokines such as IL-1 and TNF-α which stimulates the chondrocytes to produce proteolytic enzymes such as collagenase, glycohydrolases and neutral protease and degrade the cartilage which leads to diminished joint space [24]. In this study arthritic control animals showed sever inflammation with diffused joint space and bone erosion. The treatment groups showed clear joint space with no evidence of bone erosion and inflammation. Methotrexate treated group showed mild inflammation with diffused joint space. In histopathological study arthritic control showed severe joint cartilage destruction and high inflammatory cellular infiltrations. ESVM treated group showed no sign of joint destruction and cellular infiltrations. It confirmed that *V. mungo* treatment have significant cartilage protective actions.

Conclusions

The present study demonstrated that ethanolic seeds extract of *Vigna mungo* (ESVM) exhibits significant anti-arthritic activity in the CFA-induced arthritis model. Treatment with ESVM significantly reduced paw edema and arthritic index, improved body weight, and normalized hematological alterations. Biochemical assays demonstrated reduced levels of CRP, rheumatoid factor, and pro-inflammatory cytokines (TNF-α, IL-1β, IL-6). Histopathological evaluation further confirmed joint protection through reduced synovial hyperplasia, pannus formation, and cartilage destruction. These effects were dose-dependent, with higher efficacy observed at 400 mg/kg, comparable to methotrexate. These effects are likely mediated by the phytoconstituents of *Vigna mungo*, particularly flavonoids and phenolic compounds, which possess antioxidant, anti-inflammatory, and immunomodulatory properties. However, further studies involving isolation of active principles, detailed mechanistic exploration, chronic toxicity assessments, and clinical evaluation are warranted before therapeutic application in humans.

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