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IN-VIVO ANTI-ARTHRITIC EFFECT OF ETHANOL EXTRACT OF *SESBANIA GRANDIFLORA* SEEDS ON COMPLETE FREUND'S ADJUVANT (CFA)-INDUCED ARTHRITIS IN RATS

S. N. Pandey and B. C. Semwal *

Department of Pharmacology, Institute of Pharmaceutical Research GLA University, Mathura - 281406, Uttar Pradesh, India.

Keywords:

Sesbania grandiflora, UK-356618, CFA, Arthritis, Hemoglobin

Correspondence to Author:
Bhupesh Chandra Semwal

Assistant Professor,
Department of Pharmacology
Institute of Pharmaceutical Research GLA University, Mathura,
NH#2 Delhi Mathura Highway P.O. Chaumuhan – 281406, Uttar Pradesh, India.

E-mail: bhupesh115@gmail.com

ABSTRACT: Herbal medicines are in substantial demand in the developed country for primary healthcare because of their high efficacy, safety, and few side effects. *Sesbania grandiflora* is an ancient plant that belongs to the family Fabaceae. Traditionally, it is used for the treatment of various disorders such as diabetes, anxiety, hepatotoxicity, ulcer, and anti-inflammatory activity. Therefore the present investigation was undertaken to investigate the anti-arthritic potential of *Sesbania grandiflora* seeds extract against CFA-induced arthritis in experimental animals. The Ethanol extract of *Sesbania grandiflora* seeds was prepared by liquid-liquid extraction procedure, and phytochemical analysis of extract was done by the different chemical test as well as HPLC. Daily oral treatment of ethanol extract of *Sesbania grandiflora* and UK-356618 for twenty one days. After nine day of CFA administration improve the body weight and attenuated the paw volume, hepatic biomarkers as well as hematological parameters such as hemoglobin, RBC, Hb, WBC, and ESR of experimental animals. Finding of the current investigation demonstrated that the ethanol extract of *Sesbania grandiflora* along with UK-356618 possesses potent anti-arthritic activity; further investigations are required to explore the mechanism responsible for its anti-arthritic activity.

INTRODUCTION: Rheumatoid arthritis (RA) lifelong progressive inflammatory, autoimmune disorders that affect worldwide approximately 1% adult population [1] characterized by swelling, joint pain, pannus formation, destruction of joint, impaired functions, and increase morbidity and mortality [2]. The Prevalence is found to be two to three times higher in women over the age of 40 years than the men [3].

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Immune cells stimulate the production of various pro-inflammatory markers such as tumor necrosis factor-α (TNF-α) and interleukin (IL-6) and increase the severity of disease [4]. The patho-physiology of RA involves several pathways such as mitogen-activated protein kinase (MAPK), nuclear factor-kappaB, and Janus kinase (JAK)-signal transducer and activator of transcription (JAK/STAT pathway [5, 6].

Disease-modifying anti-rheumatic drugs, gluco-corticoid, and nonsteroidal anti-inflammatory drugs are the first-line drug therapy used in the treatment of arthritis [7]. However, prolong use of gluco-corticoids inhibits the synthesis of tissue inhibitors of metalloproteinases (TIMP-1) and increases the expression of MMP-3 and MMP-9 [8].

MMPs have been considered as an outstanding target for the treatment of several diseases, including arthritis; various clinical and animal studies suggested that inhibition of MMP-3 activity in various disease conditions decreased the severity of disease and improved the functional activity of joints [9]. Moreover, the therapeutic use of MMP-3 inhibitors are limited for the treatment of arthritis due to its dose and musculoskeletal side effects in human beings such as stiffness, inflammation, and pain in the hands and legs [10].

In India, numerous ayurvedic specialists utilize different indigenous plants for the treatment of various kinds of joint disorders, and these drugs can effectively decrease the inflammatory response safely and offer an alternative drug of choice to patients. *Sesbania grandiflora*, also named as Agastya (Fabaceae) and it is a fast, growing, loosely branched plant that grown up to 8- 15 m (l) and 25-30 cm (d) [11]. The whole plant is used for edible purposes due to its high nutritional value. Traditionally, it is used in the treatment of various disorders such as cough, CNS disorder, cold, indigestion, pain, and inflammation [12].

Based on the literature review on *S. grandiflora*, which yielded positive anti-inflammatory results, the present study has been designed to investigate the anti-arthritic potential of *S. grandiflora* seeds extract on CFA-induced arthritis in experimental animals.

Drugs and Chemicals: CFA was purchased from Sigma Aldrich (St. Louis, Missouri, United States). AST, ALT and Total protein kit were purchased from Auto span Pvt. Ltd. All other reagents and chemicals like ethanol, chloroform, and petroleum ether were purchased from local suppliers which are of analytical grade

MATERIALS AND METHODS: Dried seeds of *Sesbania grandiflora* were purchased from the Amazon E-commerce company and authentified (Ref. No- NISCAIR/RHMD/Consult/2019/3434-35) by Dr. Sunita Garg, Chief Scientist, CSIR-NISCAIR, New Delhi. The dried seeds were powdered in the grinder and soaked in ethanol and water (80: 20) for triple maceration. The extracts were filtered and concentrated by distilling off the solvent under reduced pressure at 40 °C using a rotary evaporator. The dried extract was further dissolved in water and then extracted with petroleum ether, chloroform, and ethanol through liquid-liquid extraction based on increasing polarity [14]. After filtration, each extract was dried with a hicom rotary evaporator, and ethanol extract was selected for investigation of anti-arthritic potential in Sprague dwely rats.

Determination of Total Phenolic and Flavonoid Content: The presence of total phenolic content in Ethanol extract of *Sesbania grandiflora* (EESG) was determined by using Folin ciocalteu reagent [15] with a slight change in the assay procedure. Gallic acid was taken as a standard, and absorbance of the test solution was taken in Double-Beam UV-Vis Spectrophotometer at λ_{max} 725 nm. The amount of total polyphenols was expressed as milligrams of gallic acid equivalents (mg GAE/g).

The flavonoid content in EESG was determined in colorimetry by using $AlCl_3$ [16]. Quercetin was used as a standard, and absorbance of the test solution was recorded in Double-Beam UV-Vis Spectro-photometer at 420 nm. The quantity of total flavonoids was expressed as quercetin equivalents (mg quercetin/g extract).

HPLC Analysis: The ethanol extract fraction of *Sesbania grandiflora* extract was analyzed for the estimation of flavonoid content using Agilent HPLC 1260 Infinity II quaternary pump VL system with photodiode array detector and carried out to according to the previously established method of Gupta *et al.,* [17] Separation was done on a particle size Poroshell 120 EC-C18, 4.6 × 100 mm, 4 µm column and gradient run under reverse-phase partition chromatographic condition. The equipment was controlled by a PC with properly installed chromatographic software. Different mobile phase compositions were tried for the optimization of the method. The analysis was done using mobile phase Acetonitrile: 1% ortho-phosphoric acid (80:20) at the 280 nm wavelength with 1 mL/min flow rate. Standard and sample solution was filtered properly before injecting in an instrument with membrane filtration assembly.

Animals: Healthy Sprague-Dawley female rats weighing 150-180 g, were housed in the poly-propylene cages under standard condition12 h light

and 12 h dark cycle and allowed to free access to food (Aishrwad) and water. Animal experimental protocol was approved (CPCSEA/IAEC/2019/ P.Col/R12) by the Institutional animal ethical committee of the Institute of Pharmaceutical Research, GLA University, Mathura, India.

Animals and Experimental Design: Whole experimental protocol was designed for 30 days, and animals were divided into different groups of six animals in each group. Group I and II served as Normal and CFA-control and received normal saline; group III, given methotrexate (5 mg/kg) 17 IV, V and VI received 800 mg/kg of *Sesbania grandiflora* ethanol extract and MMP-3 inhibitor (UK-356618)18 in alone and in combination.

Induction of Arthritis: The experimental protocol used in the present investigation by the procedure described by Voon *et al.* [19] The detailed protocol is shown in **Fig. 1**. Briefly, 0.1 mL of CFA was injected into the sub plantar region of the left hind paw of an individual animal under light anesthesia (Thiopental sodium).

The time of adjuvant injection was referred to as day 0. Nine days after the induction of arthritis, daily oral treatments were started for 21 days. On the last day, one hour after the treatment, the animals were sacrificed, and blood samples were collected *via* cardiac puncture.

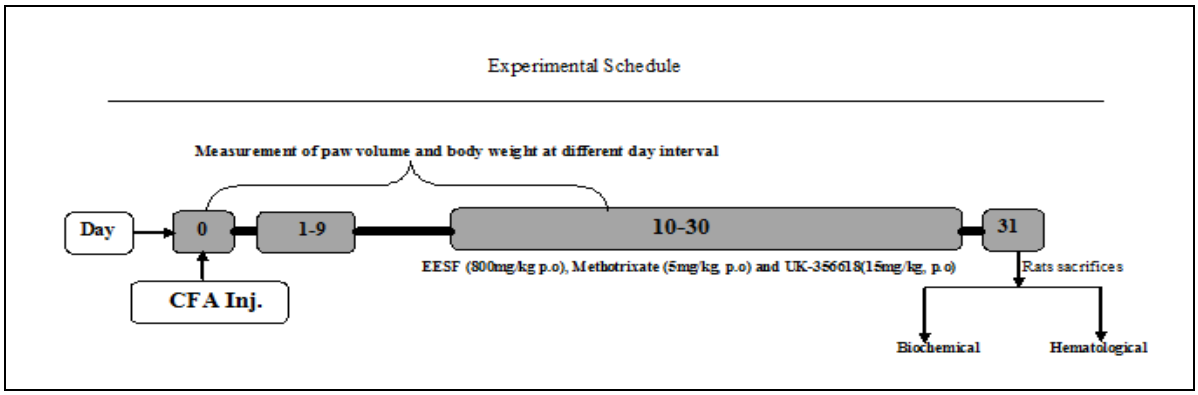

FIG. 1: DIAGRAMMATIC REPRESENTATION OF DETAILED EXPERIMENTAL SCHEDULE

Assessment of Arthritis: The paw volume of each animal was measured before CFA injection using a digital plethysmometer (Rolex India). Further, after administration of CFA body weight and paw volume of each animal were measured at different day's interval 10, 15, 20, 25 and 30 [20].

Biochemical and Hematological Analysis: On the last day of the experiment blood sample of each animal was collected in heparinized test tubes by cardiac puncture and allowed to stand for 30 min to separate the serum.

The serum was then centrifuged at 4000 rpm, after which total RBC, WBC was determined by hemocytometer, ESR and percentage of hemo-globin was measured by westergren and sahlis method. SGOT, SGPT, and Total protein were examined by a commercially available kit.

Statistical Analysis: The results were expressed as mean ± S.E.M. Statistical analysis was performed using Graph Pad Prism 5.2® software.

Two-way analyses of variances (ANOVA) were performed on percentage inhibition of paw volume and effect on weight variation in arthritic rats, followed by Turkey's post hoc test.

The effect of ethanol extract and UK-356618 on the arthritic score of the rats, determined on different days, were analyzed by the Chi-square test. All other data were analyzed by one-way ANOVA followed by Turkey's test.

RESULT AND DISCUSSION:

TABLE 1: PHYTOCHEMICAL ANALYSIS OF DIFFERENT EXTRACT OF *SESBANIA GRANDIFLORA* SEEDS

S. no	Phytoconstituents	Petroleum Ether	Chloroform	Ethanol
1	Alkaloids	--	+	++
2	Carbohydrates	+	--	++
3	Proteins	--	--	++
4	Tannins & polyphenols	--	++	++
5	Flavonoids	--	+	++
6	Glycosides	--	--	+
7	Steroids	++	+	+

TFC and TPC: The ethanol extract of *Sesbania grandiflora* was evaluated for total phenolic and total flavonoid content.

The EESG showed (80.28 ± 1.51 mg GAE/g) and (45.25 ± 2.20 mg RE/g).

HPLC: The HPLC chromatogram of ethanol extract of *Sesbania grandiflora* seeds extract depicted in **Fig. 2A** and **2B**. HPLC analysis of ethanol extract revealed the presence of quercetin. The chromatogram of extract was matched with the standard.

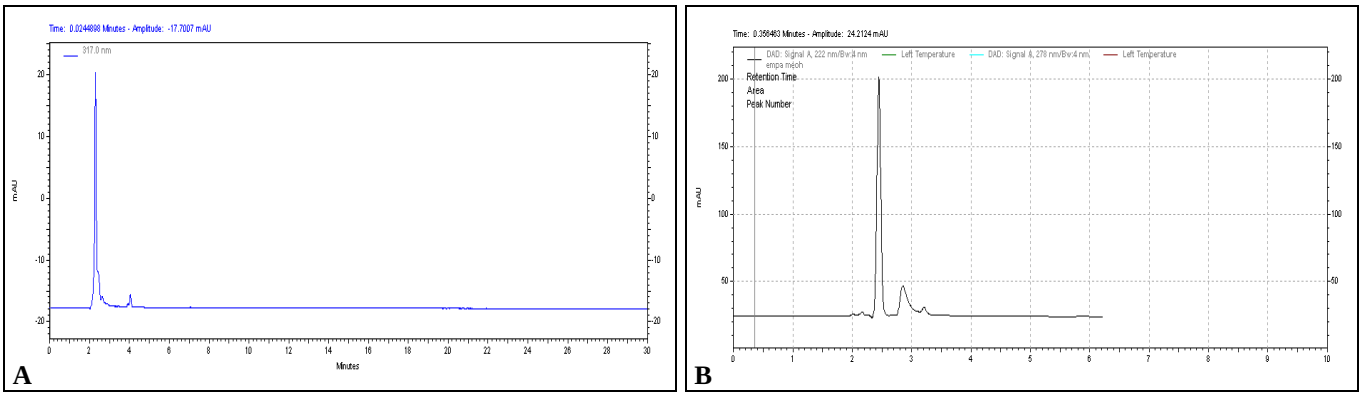

FIG. 2: HPLC CHROMATOGRAMS OF STANDARD QUERCETIN (A) AND EESG (B)

Effect on Bodyweight: The changes in body weight of normal, CFA control, methotrexate (5 mg/kg), EESG (800 mg/kg), UK-356618 (15 mg/kg), and combination of EESG and UK-356618 treated arthritic rats before and after the injection of CFA are depicted in **Fig. 3**. A loss in body weight was observed in all the experimental animals treated with CFA compared to healthy animals. Treatment of EESG 800 mg/kg significantly ($p<0.05$) improved the loss of body weight caused by CFA. However, treatment of methotrexate did not show any remarkable effect on loss of body weight compared to EESG. However, treatment of EESG and UK-356618 in combination significantly attenuated the loss of body weight compared to EESG and UK-356618 alone therapy.

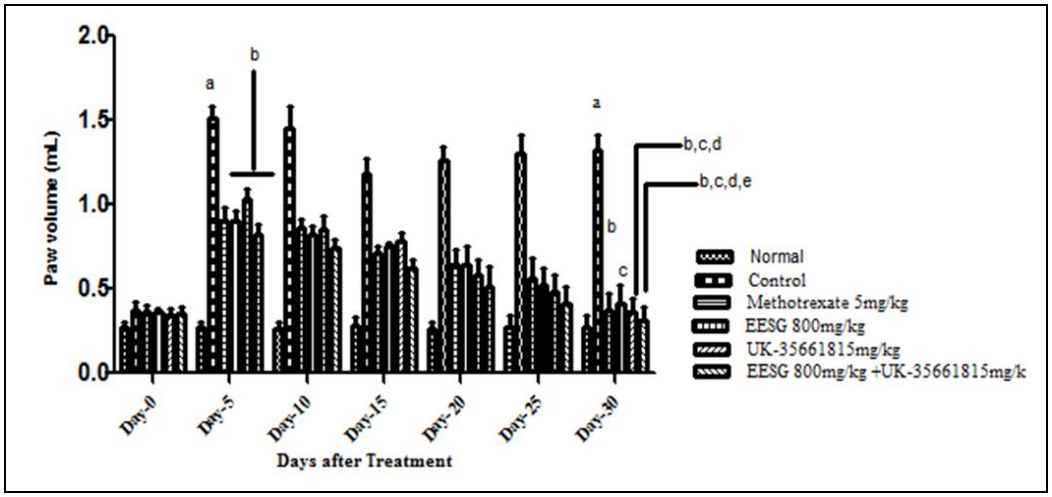

FIG. 3: EFFECT OF *SESBANIA GRANDIFLORA* SEEDS EXTRACT ON CFA-INDUCED CHANGES IN BODY WEIGHT. All values are mean ± SEM (n=6). *p<0.05, ***p<0.001 compared to control group of animals (Two-way ANOVA followed by Student Newmann keuls Post-hoc test).

Effect on Paw Swelling: The outcome of EESG on CFA-induced paw swelling is depicted in **Fig. 4** Sub plantar injection of CFA significantly ($p<0.001$) increased the swelling of the paw in experimental animals. Daily treatment of EESG, UK-356618 and methotrexate for twenty-one days after nine-day of CFA injection attenuated the CFA-induced paw volume ($p<0.001$) in experimental animals compared to the disease control group. Moreover, treatment of EESG and UK-356618 in combination exhibited a maximum of reduction in paw swelling compared to alone.

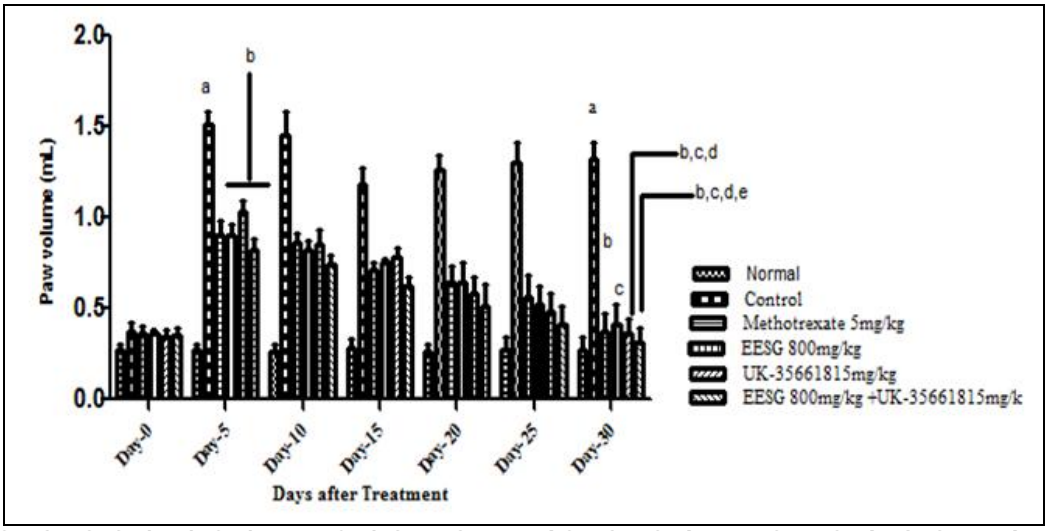

FIG. 4: EFFECT OF *SESBANIA GRANDIFLORA* SEEDS EXTRACT ON CFA-INDUCED CHANGES IN PAW VOLUME. All values are mean ± SEM (n=6). ap<0.05 compared to Control, bp<0.05 compared to Disease control, cp<0.05 compared to Methotrixate, dp<0.05 compared to EESG, ep<0.05 compared to UK35661815 (One-way ANOVA followed by Student Newmann keuls Post-hoc test)

Estimation of Biochemical Parameters:

Effect on AST and ALT: Fig. 5 illustrates the response of EESG on AST (a) and ALT (b). Serum aspartate aminotransferase and alanine amino-transferase are sensitive indicators of liver injury. The administration of CFA significantly increased the concentration of AST and ALT in all the groups of animals compared to healthy animals. Treatment of ethanol extract of EESG seeds showed a significant (p<0.05) reduction in elevated levels of AST and ALT against the CFA induced arthritic group. However, the treatment of methotrexate did not show much more remarkable effect on hepatic biomarker compared to ethanol extract of *Sesbania grandiflora* and UK-356618 treated group. Moreover, treatment of *Sesbania grandiflora* and UK-356618 in combination showed a remarkable reduction in elevated levels of AST and ALT compared to alone treated animals.

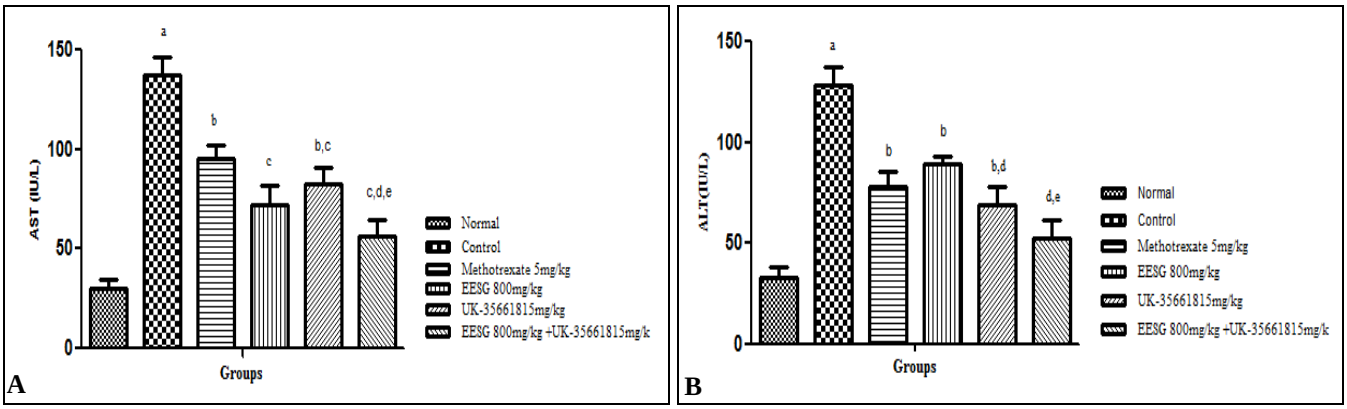

FIG. 5: EFFECT OF *SESBANIA GRANDIFLORA* SEEDS EXTRACT ON CFA-INDUCED CHANGES IN AST AND ALT LEVEL. All values are mean ± SEM (n=6). ap<0.05 compared to Control, bp<0.05 compared to Disease control, cp<0.05 compared to Methotrixate, dp<0.05 compared to EESG, ep<0.05 compared to UK35661815 (One-way ANOVA followed by Student Newmann keuls Post-hoc test).

Effect of *Sesbania grandiflora* Seeds Extract on Serum Total Protein: The response of EESG on total protein is depicted in **Fig. 6**. In CFA-induced arthritis, the total serum protein level decreased in all the experimental animals compared to healthy animals. Statistical analysis revealed that repeated treatment of EESG, methotrexate, and UK-356618 treated rats showed a remarkable increment in their total serum protein compared to the diseased control group. However, treatment of EESG and UK-356618 in combination showed a significant increment in their level of serum protein compared to methotrexate and UK-356618 alone.

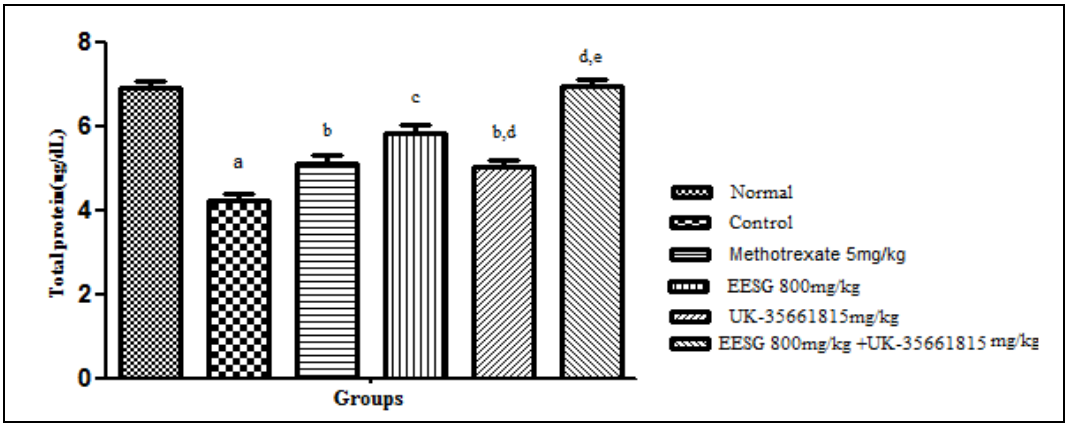

FIG. 6: EFFECT OF *SESBANIA GRANDIFLORA* SEEDS EXTRACT ON CFA-INDUCED CHANGES IN TOTAL PROTEIN LEVEL. All values are mean ± SEM (n=6). [a]p<0.05 compared to Control, bp<0.05 compared to Disease control, cp<0.05 compared to Methotrixate, dp<0.05 compared to EESG, ep<0.05 compared to UK35661815 (One-way ANOVA followed by Student Newmann keuls Post-hoc test)

Hematological Parameters: The effect of EESG on RBC, WBC, Hb, and ESR of CFA- induced arthritic animals is depicted in **Table 2**. The Hematological parameters, including RBC, WBC, and hemoglobin and erythrocyte sedimentation rate, were decreased after CFA administration. There were significant decrease in RBC from 5.43 ± 0.33 to 3.62 ± 0.32 mm^3, Hb from 13.65 ± 0.31 to 7.4 ± 0.86 g/dL and increase in WBC from 7014 ± 4.2 to 8754 ± 8.26 and ESR from 2.32 ± 0.07 to 7.12 ± 0.08 mm/h. Treatment of EESG reverses the elevated level of WBC and ESR and boost up the Hb and RBC count, all most up to normal range in diseased animals. Moreover, the treatment of methotrexate and UK-356618 significantly decreased the elevated level of ESR and WBC count but did not show any remarkable effect on RBC count and hemoglobin level. However, *Sesbania grandiflora* and UK-356618 treatment show a better effect on all the hematological profiles compared to methotrexate, *Sesbania grandiflora*, and UK-356618 alone.

TABLE 2: EFFECT OF EESG ON HEMATOLOGICAL PARAMETERS

Groups	RBC mm^3	Hb% g/dl	WBC mm^3	ESR
Normal	5.43 ± 0.33	13.65 ± 0.31	7014 ± 4.2	2.32 ± 0.07
CFA	3.62 ± 0.3	8.9 ± 0.86	8754 ± 8.26	7.12 ± 0.08
CFA + Methotrixate 0 mg/kg	4.20 ± 0.55	10.17 ± 0.37	7036 ± 6.6	3.09 ± 0.07
CFA + EESG 800 mg/kg	4.46 ± 0.33	11.87 ± 0.71	7068 ± 6.45	3.36 ± 0.21
CFA + UK-356618	3.82 ± 0.30	9.6 ± 0.86a	7018 ± 7.16	3.64 ± 0.04
CFA + EESG+ UK-356618	4.68 ± 0.41	12.76 ± 0.46	7029 ± 5.88	2.73 ± 0.09

DISCUSSION: Medicines have a crucial role in the treatment of poor health of humans since the historical items and herbal drugs are commonly used as a folk medicine or in clinics for the relief of arthralgia. These drugs can not only prevent the inflamed joint from bone destruction, but in comparison to modern medicine, these are safe, acceptable and suitable for patients [21, 22]. The present study for the first time demonstrates that ethanol extract of *Sesbania grandiflora* seeds along with MMP-3 inhibitor attenuated the CFA induced RA in Sprague-Dawley rats. Therefore ethanol extract of *Sesbania grandiflora* could be a potential therapeutic alternative in the treatment of RA. The rodent model CFA-induced arthritis was selected for the present investigation.

CFA-induced arthritis elicits similar symptoms of RA as occurs in humans, such as pain, redness, and swelling [23]. Intradermal injection of CFA substantially increases the paw volume in Sprague-Dawley rats, similar to earlier findings [24]. The result of the present study showed that the treatment of EESG significantly decreases the paw volume in experimental animals that demonstrated a reduction in the growth of the disease.

It is believed that loss of body weight occurs in RA, and it is directly related to the severity of disease [25]. Weight losses may probably occur due to loss of appetite, defect in the absorption of nutrients from the intestine or increased metabolism of amino acids [26].

The results of the current investigation indicate that there is a close connection between the loss of body weight and inflammation of joints. Treatment of EESG attenuated the loss of body weight in CFA-induced arthritic animals, which could be due to improvement in the absorption of nutrition's from the intestine or presence of phytochemicals such as flavonoids which decrease the catabolic activity of amino acids and thus prevents the loss of body weight.

Further, the present study also revealed that the treatment of *Sesbania grandiflora* seeds extracts along with MMP-3 inhibitor significantly increased the pain threshold and motor activity against the CFA-induced arthritic animals.

It has been suggested from experimental studies that the level of MMP-3 increased in arthritis as the severity of disease increased [27]. Our result demonstrated that the treatment of *Sesbania grandiflora* and MMP-3 inhibitor simultaneously inhibits pain sensation and improved the motor activity of experimental animals, probably due to inhibition of cytokines release.

As pieces of evidence documented that flavonoids and phenolic compounds are potent antioxidant enzymes that can decrease tissue injury [28]. *Sesbania grandiflora* contains various phenolic compounds (Gallic acid) and flavanoid (quercetin), which may be responsible for the synergistic effect of *Sesbania grandiflora*.

Various scientists have proposed that the estimation of liver biomarkers such as SGOT, SGPT, and total protein is an excellent approach to measure the anti-arthritic potential of test drugs [29, 30]. In the current investigation, CFA caused a significant elevation in the level of SGOT and SGPT and a reduction in total protein concentration similar to that of earlier findings [31].

In the present study, *Sesbania grandiflora* and MMP-3 inhibitor diminished the CFA-induced elevated concentration of SGOT and SGPT and reversed the diminished concentration of protein to a normal level. From the result of the present, it can be assumed that the presence of different flavonoids could be responsible for attenuation of the elevated level of the hepatic marker, because these flavanoids are potent antioxidants [32] which may control the activity of MMP and regulate the production of MMP and TIMP ratio. In the present study, CFA caused a reduction in the level of hemoglobin and red blood cell count.

A low level of hemoglobin represents the anemic condition may be due to the inadequate absorption of iron [33]. Oral treatment of *Sesbania grandiflora* seeds extract significantly increased the percentage of hemoglobin and RBC count in diseased animals.

However, the elevation in white blood cell counts might be due to the stimulation of the immune system [34]. Treatment of *Sesbania grandiflora* decreased the total leukocyte count in experimental animals could be due to the immunomodulatory effect of *Sesbania grandiflora* [35] and MMP-3 inhibitor.

CONCLUSION: The present study revealed that repeated treatment of EESG either alone or in combination with UK-356618 significantly decreased symptoms of arthritis such as paw volume in bodyweight and hepatic markers in the CFA-induced arthritic animals.

The symptomatic relief in the present study might be due to the presence of flavonoid (quercetin) or any other phytoconstituents present in the extract. Further study is required to confirm the antiarthritic potential of the extract.

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Both the authors contributed equally to this work. B.C. Semwal developed the concept, designed the experiment, and prepared the manuscript. S.N. Pandey performed the experiment.

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