IJPSR (2016), Vol. 7, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 April, 2016; received in revised form, 17 July, 2016; accepted, 27 July, 2016; published 01 September, 2016

EVALUATION OF ANTI-ARTHRITIC POTENTIAL OF THE HYDRO-ALCOHOLIC EXTRACT OF THE STEM BARK OF *PLUMERIA RUBRA IN* FREUND'S COMPLETE ADJUVANT-INDUCED ARTHRITIS IN RATS.

Prodyut Mondal, Sonjit Das, Kabita Mahato, Sudarshana Borah, Julfikar Ali Junejo and Kamaruz Zaman*

Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India.

Keywords:

Anti-arthritis, *Plumeria rubra*, inflammation, Phytochemical, complete Freund's adjuvant

Correspondence to Author:
Kamaruz Zaman

Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India.

E-mail: z_kamar2003@yahoo.com

ABSTRACT: To investigate the anti-arthritis effects of hydro-alcoholic stem bark extract (HSBE) of *Plumeria rubra* in Complete Freund's adjuvant (CFA) induced arthritis model in wistar albino rats. Paw edema was produced by sub-plantar injection of 0.1 ml of complete Freund's adjuvant (CFA) and paw volume was measured with plethysmometer. HSBE of the plant *P. rubra* (250 mg/kg & 500 mg/kg body weight) was administered orally for 14 days. Arthritic assessment was carried out based on parameters including paw edema, body weight, and spleen index. At the end of study period, animals were sacrificed and various biochemical, oxidative stress parameters, radiological and histological parameters were evaluated. Administration of HSBE significantly attenuated the behavioral, biochemical, hematological, radiological alteration induced by the CFA in dose dependent manner. From our study, we can conclude that the hydro-alcoholic extract of the plant has the potent activity to check the arthritis edema as well as inflammation & body weight. The potency of ant-oxidant activity was managed significantly. The radiological and histopathological investigations revealed that the extract produce significant reduction in mononuclear infiltration and bone erosion showed against adjuvant induced arthritis.

INTRODUCTION: Arthritis is a musculoskeletal system disorder that destabilizes normal coupling between degradation and regeneration of articular cartilage in joints following mechanical and a biological collapse in the body. This chronic disease is a chief cause of turmoil in the daily life of mankind throughout the world. The pathophysiological phenomenon of arthritis involves up-regulation of pro-inflammatory cytokines and over expression of pro-inflammatory enzymes, resulting in elevated levels of prostaglandins, leukotrienes and nitric oxide [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(9).3675-88

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (9).3675-88

Arthritis can affect individuals of any age, ranging from 25 to 50 years, predominantly among the geriatric people. There are about 100 types of arthritis of which the most commonly occurring include osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematous and juvenile arthritis [2].

Rheumatoid arthritis (RA) is a common, chronic, inflammatory, autoimmune disease of unknown etiology affecting approximately 1% of the world's population [3]. About, 0.75% of the adult Indian populations are affected by this disease [4]. It negatively affects patient's quality of life, normal work capability, and life expectancy. Besides the consequences on the health status of individuals, RA has a substantial economic impact on patients, their family and society. The mortality rate in patients with RA is increasing in comparison to the general population [5]. Presently, for the treatment of

rheumatoid arthritis generally four types of drugs are prescribed as non-steroidal anti-inflammatory drugs (NSAIDs such as diclofenac sodium, ibuprofen), steroids hormone (as dexamethasone), disease modifying anti-rheumatic drugs (DMARDs such as methotrexate, cyclosporine) and immunosuppressant. According to the guidelines of the American College of Rheuma-tology, newly diagnosed RA patients were strongly recommended to begin treatment with NSAIDs for relieving noceceptive pain and controlling inflammation, with combined use of DMARDs for reducing the disease activity, preventing joint deformity and improving joint function [6, 7].

However, these synthetic molecules have not been proven to provide satisfactory treatment management due to toxicity, side effects, drug resistance or recurrence of symptoms on discontinuation. Administration of these drugs are known to produce various side effects including gastrointestinal disorders, immunodeficiency and humoral disturbances, cardiovascular complications etc [8]. Moreover, the anti-arthritic drugs which inhibits the pro-inflammatory markers, like tumor necrosis factors (TNF- α),interleukin-1 (IL-1) and interleukin-6 (IL-6) has life threatening side effects, and their long-term risk is not very much known, hence, limiting their clinical use for long-term basis [9, 10].

Hence, there is an urgent need to identify an alternative treatment options for arthritis. There is a continuous search for alternative safe and effective anti arthritic agents is persisting especially from natural sources [11]. A large flora of herbal medicines are available in traditional folk medicine. Basic as well as applied scientific research has provided an understanding of the efficacy of these remedies in controlling arthritis. In recent years, there has been a boom in research and industries, focusing on herbal remedies to treat a variety of diseases including arthritis. Several developments in well designed clinical studies and trials have made the way easier to include herbs for safe and effective arthritis therapy [12].

Amongst the various experimental animal models of arthritis, complete Freund's adjuvant (CFA) induced poly-arthritis is one of the established methods which mimics the human pathophysiological state, including chronic swelling in multiple joints due to accumulation of inflammatory cells, joint cartilage erosion and bone destruction. CFA, induces arthritis through altered leukocyte proliferation and differentiation, secretion of cytokines by mononuclear phagocytes, and transient activation and proliferation of CD41 lymphocytes [13]. The plant *Plumeria rubra* (PR) is grown abundantly in the North-East region & throughout of India.

The flower of the *Plumeria rubra***,** is used as holistic purposesand its' common name is "Gour-Champa". The plant traditionally has great importance, the bark & leaves extract was found to be effective to treat the acute cases of rheumatoid arthritis, severe joint pain etc. as per folk medicinal uses [14, 15]. Traditional reports suggest that it possesses anti-inflammatory and analgesic activity. Hence, aim of the present investigation was to evaluate the anti-arthritic potential of hydro-alcoholic stem bark extract (HSBE) of *Plumeria rubra* in CFA induced arthritis models in laboratory animals based on various behavioral, biochemical and histopathological parameters. This study was undertaken to validate the traditional claim of *Plumeria rubra*as scientifically. The present study was conducted with the alkaloid and flavonoid enriched hydro-alcoholic stem bark extract to evaluate the anti-arthritic potential of *P.rubra*. Because the alkaloid, and flavonoid like compounds possess promising anti-arthritic activity in various auto-immune disease model as supported by earlier reports [16].

2. MATERIALS & METHODS:

2.1 Plant material: The fresh collected bark had air dried in the laboratory. The specimen has authenticated by the Botanical Survey of India, Shilong and a voucher specimen (Specimen No-DU/PM/2012/8) has been kept in our departmental library for future references. After authentication, fresh barks were processed in bulk, cleaned, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

2.2 Preparation of extract:

The powdered bark was extracted with ethanol and water (3:7) by cold maceration process. The extract

was then filtered and evaporated to dryness at -40°C under reduce pressure in a lyophilizer. The dark brown sticky mass was kept in a desicator and stored at 2-8^{0}C.

2.3 Preliminary Phytochemical screening:

Plumeria rubra is an evergreen plant found throughout India, especially in Himalayas, Kerala, Bengal and whole eastern region. Qualitative phytochemical investigation of methanolic and hydro-alcholic steam bark extract was carried out using standard test of different phytochemicals e.g. alkaloids, glycosides, flavonoids etc.

2.4 Animal care and maintenance:

Experiments were performed with 6-8 weeks old, healthy, male albino wistar rats, of body weight 150-250 g. Rats were housed under standard environmental conditions (25 ± 2º C temperature, 50 ± 5% humidity with a 12:12 hr. dark and light cycle) and maintained with free access of water and a standard pellet (Lipton India. Ltd.). The animals were used with the approval protocol of the Institute Animal Ethics Committee (Approval no. IAEC/DU/37 Dated: 03/12/2012. Regd. No. 1576/Go/a/11/CPCSEA dated 17.02.2012). Animals described as fasted were deprived of food for 16 hours but had free access to water.

2.5 Acute toxicity studies:

An acute toxicity study was carried out for the HSBE Extract was dissolved in 0.3% w/v tween 20. Graded doses of 250 mg/kg & 500 mg/kg body weight were chosen based on acute toxicity studies done in Wistar albino rat using OECD guidelines [17]. The HSBE extract was administered orally. The animals were monitored continuously for two consecutive weeks following administration. Body weight, food consumption, fluid intake and psychomotor activities were recorded daily.

2.6 Experimental Procedure:

Complete Freund's adjuvant induced paw edema in rats. The animals were divided into five groups of six animals each as follows:

Group I: Normal control: received 1% aqueous solution of Tween- 20 per oral (p.o.)

Group II: Disease control animals: received 0.1ml CFA (6 mg/ml) tween solution.

Group III: Standard treated animals: received Piroxicam (10 mg/kg, p.o.)

Group IV: Drug treated animals: received HSBE (250 mg/kg, p.o.)

Group V: Drug treated animals: received HSBE (500 mg/kg, p.o.)

Experimental arthritis was induced in rats according to the method proposed by Quan et al., 2008 [18]. Each rat was injected with 0.1 ml of Freund's complete adjuvant (FCA) in to subplantar region of left hind paw on day one under light ether anesthesia. Anti-arthritic activity of HSBE was evaluated on joint diameter, paw volume, and arthritic score on day 0, 1, 3, 5, 7, 9, 11, 13 and day 14. On day 14th, blood was withdrawn by retro-orbital puncture for assessment of biochemical parameter and animals were sacrificed to study the joint histology.

2.7 Arthritic score:

The arthritis morphological feature of like redness, swelling and erythema [19], was monitored by set visual criteria as follows: normal paw = 0, mild swelling and erythema of digits = 1, swelling and erythema of the digits = 2, severe swelling and erythema = 3, gross deformity and inability to use the limb = 4 on respective days.

2.8 Paw volume:

The hind paw volumes of all animals were measured just before CFA injection on day 0 and thereafter at definite intervals till day 14 using a plethysmometer [20]. The change in paw volume was measured as the difference between the final and initial paw volumes.

2.9 Index of immune organ (spleen):

At the end of the experiment, rats were sacrificed by cervical dislocation & spleens were removed. All the spleens of rats were weighed immediately after dissection. The spleen indexes were calculated by using the following formula [21].

Spleen index= [weight of spleen (gms)/ body weight (gms)] X 100

2.10 Rheumatoid factor:

Rheumatoid arthritis is diagnosed by rheumatoid factor. The abnormal antibodies (IgG) which are present in blood, reacts with the antigen and form antigen-antibody complex that leads to pain and inflammation of synovial membrane. The latex turbidimetry method was used in the present study using RF turbilatex kit of Beacon Company Pvt. Ltd. Calibration was carried out for linear range up to 100 IU/mL. The reading of RF factor of all the groups obtained was compared with the control Animals and was expressed as IU/ml [22].

2.11 Measurement of inflammatory mediators:

Lipid peroxidation was estimated by measuring thiobarbituric acid reacting substances (TBARS). The method is based on spectrometric measurement of purple color generated by the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA). The concentration of MDA was calculated based on the absorbance coefficient of TBA-MDA complex and it was expressed as nmol MDA/mg of protein [23]. Catalase activity was measured based on the ability of the enzyme to break down H_2O_2. A unit catalase activity is the amount of enzyme that liberates half the peroxide oxygen from an H_2O_2 solution of any concentration in 100 seconds at 25^0C [24]. The activity was expressed as μM of H_2O_2 formed/min/g. Reduced Glutathione was estimated spectrophotometrically by determination of Di-thio-bis-(2-nitrobenzoic acid (DTNB) which gets reduced by SH-groups. Reduced glutathione was determined by the method of G. L Elman [25].

2.12 Histological processing and assessment of arthritis damage:

On 14^{th} day the rats were sacrificed, knee joints were removed and fixed in 4% formaldehyde solutionfor four days. After decalcification with EDTA at 4^0C in 5 % formic acid, the specimens were processed for paraffin embedding. The tissue sections (7 μm thick) were cut and stained with haematoxylin and eosin and observed under light microscope for histopathological changes [26-27].

2.13 Drug and chemicals:

Freund's complete adjuvant (FCA) was obtained from Sigma-Aldrich Ltd. (USA). SGOT, SGPT, ALP kits were purchased from Beacon Pvt. Ltd. Piroxicam (East West Pharmaceutical Ltd., Roorkee, India). All standard chemicals used in the present study were freshly prepared and of analytical grade.

2.14 Statistical Analysis:

The results were expressed as mean ±SEM. The results of the present study were analyzed using one way ANOVA followed by post hoc Dunnett's Test computed statically by using Graph pad prism software (version 6.0) at $p<0.05$.

3. RESULTS:

3.1 Acute toxicity study:

Acute toxicity studies of the HSBE shows no signs and symptoms such as respiratory distress, convulsions, diarrhea, coma and it was found safe up to 2500 mg/kg.

3.2 Preliminary phytochemical screening:

The hydro-alcoholic stem bark extract and methanolic stem bark extracts of *P. rubra* was screened for various secondary metabolites test the data reported on **Table 1**.

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING

Sl. No.	Constituents	Methanol Extract	Hydro-alcohol Extract
1.	Alkaloids	+	+
2.	Carbohydrates	+	+
3.	Fats and oil	-	-
4.	Flavonoids	+	+
5.	Glycosides	+	+
6.	Gum	-	-
7.	Steroids/sterols	+	_
8.	Proteins	+	+
9.	Saponins	+	+
10.	Tannins and Phenolic compounds	+	+

3.3 Effect of HSBE on body weight:

There was decrease ($P < 0.001$) in body weight of control rats as compared to the normal rats after 14 days of sub plantar administration of CFA due the generation of immune response. The body weight was gradually decreased in disease control rats since 14 days. Treatment with HSBE (250 and 500 mg/kg) showed significant and dose-dependent increase of weight. The data represents in **Fig. 1**.

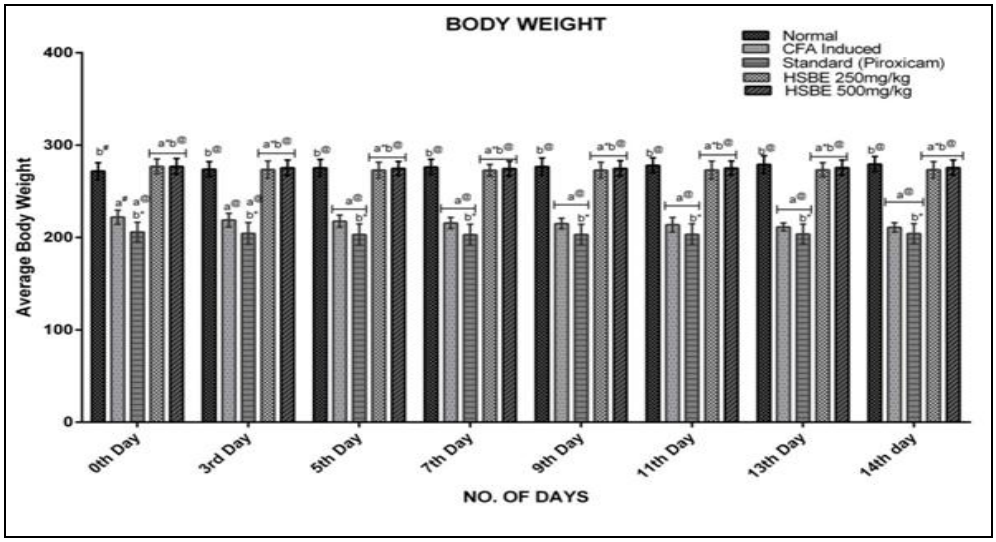

FIG 1: EFFECT OF HSBE ON BODY WEIGHT IN CFA INDUCED ARTHRITIC RATS. DATA ARE EXPRESSED AS MEAN ± SEM, n = SIX ANIMALS IN EACH GROUP.

a: Normal versus CFA Induced, Standard, HSBE 250 and HSBE 500.

b: CFA Induced versus Normal, Standard, HSBE 250 and HSBE 500.

Symbols represent statistical significance: $*P< 0.05$, $\#P< 0.01$, $@P< 0.001$

3.4 Effect of HSBE on paw volume:

The one of the important physiological parameter in case of assessment of RA is paw volume. Paw volume of all the animals in each group increase initially but decrease after treatment with standard and test drugs; but in vehicle treated remain increased. Rat treated with piroxicam (10 mg/kg-BW) significantly decreased (P<0.001) paw volume of 14 days of treatment as compared to control rats.

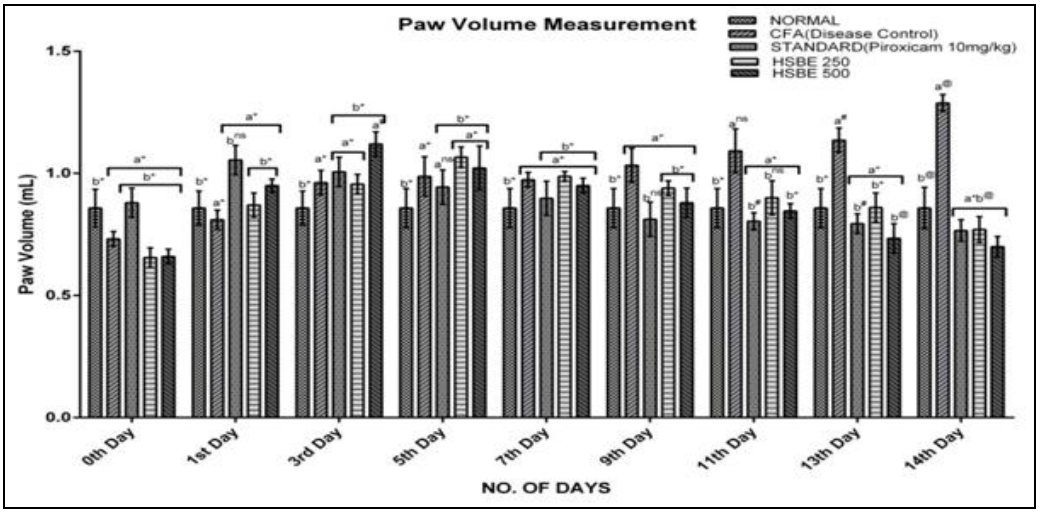

FIG 2: EFFECT OF HSBE ON PAW VOLUME IN CFA INDUCED ARTHRITIC RATS. DATA ARE EXPRESSED AS MEAN ± SEM, n = SIX ANIMALS IN EACH GROUP.

a: Normal versus CFA Induced, Standard, HSBE 250 and HSBE 500.

b : CFA Induced versus Normal, Standard, HSBE 250 and HSBE 500.

Symbols represent statistical significance: $*P< 0.05$, $\#P< 0.01$, $@P< 0.001$

3.5 Arthritic Index:

Sub plantar administration of CFA results in significant increased (P < 0.01) in arthritic index in all CFA treated rats as compared to standard and this had shown a biphasic response. Rats treated with HSBE (250 and 500 mg/kg) showed significant decreased in arthritic score (P < 0.01) when compared with CFA treated group at the end of the study.

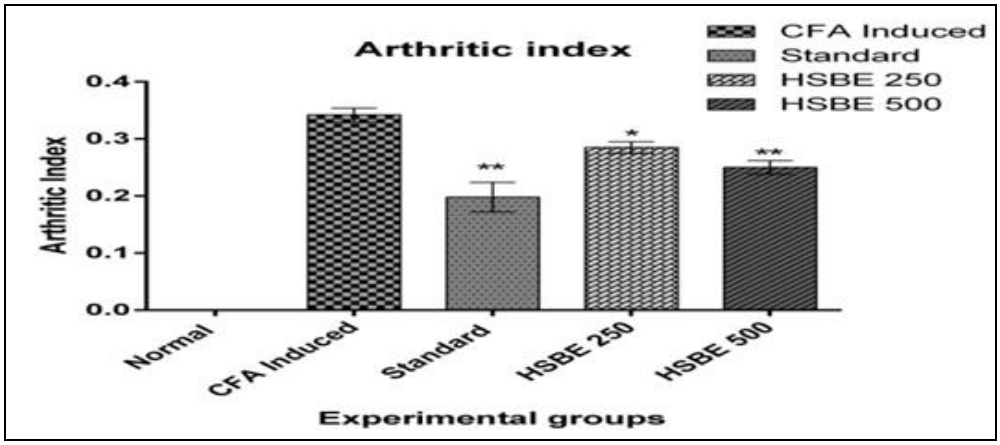

FIG.3: ARTHRITIC INDEX IN FREUND'S COMPLETE ADJUVANT INDUCED ARTHRITIS IN RATS. EACH COLUMN REPRESENTS THE MEAN ± S.E.M (n=6/GROUP). ASTERISKS DENOTE THE SIGNIFICANCE LEVELS, WHEN COMPARED TO THE CONTROL GROUP (CFA INDUCED), **P<0.01, *P<0.05.

3.6 Rheumatoid Factor:

The graphical representation of rheumatoid factor is shown on the **Fig.4**.

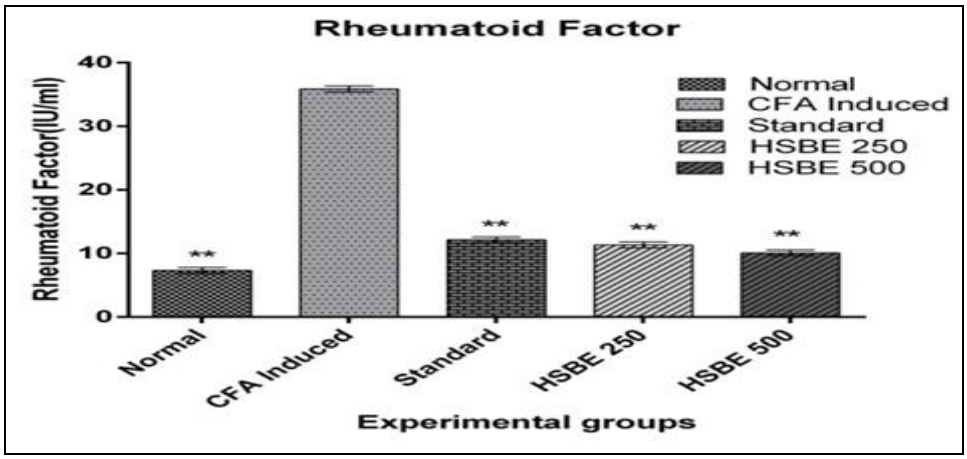

FIG 4: RHEUMATOID FACTOR IN FREUND'S COMPLETE ADJUVANT INDUCED ARTHRITIS IN RATS. EACH COLUMN REPRESENTS THE MEAN ± S.E.M (n=6/GROUP). ASTERISKS DENOTE THE SIGNIFICANCE LEVELS, WHEN COMPARED TO THE CONTROL GROUP, **P<0.01.

3.7 Spleen Index:

Fig. 5 represent the spleen index of the experimental rats. The higher dose extract treated group animals' show the higher spleen index.

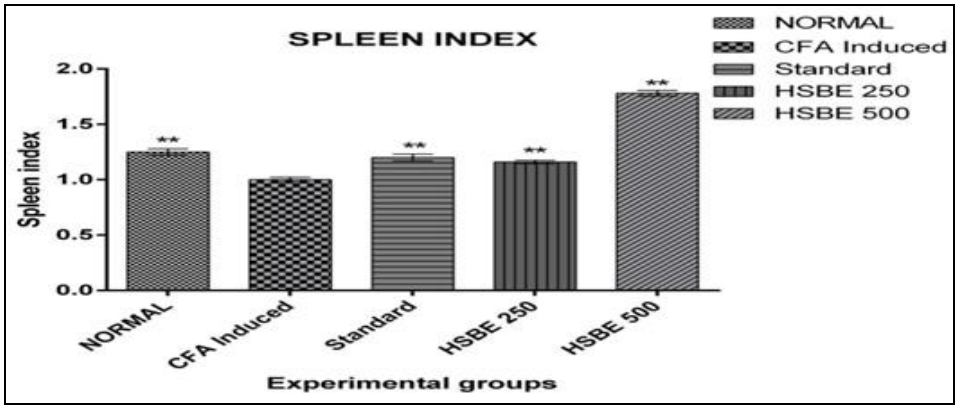

FIG. 5: SPLEEN INDEX IN FREUND'S COMPLETE ADJUVANT INDUCED ARTHRITIS IN RATS. EACH COLUMN REPRESENTS THE MEAN ± S.E.M (n=6/GROUP). ASTERISKS DENOTE THE SIGNIFICANCE LEVELS, WHEN COMPARED TO THE CONTROL GROUP (CFA INDUCED), **P<0.01.

3.8 Inflammatory mediators:

3.8.1 Lipid Peroxidation:

CFA injection produced an increased in level of tissue TBARS (Thiobarbituric acid reacting substances) expressed as nmol of MDA normal in control rats to increase in arthritic rats tissue supernatant. Figure no. 6shows the level of lipid peroxides in liver. Lipid peroxide MDA level was found to be significantly increased. After drug treatment for 14 days, the level was found to be significantly reduced with HSBE dose dependent reduction in MDA level.

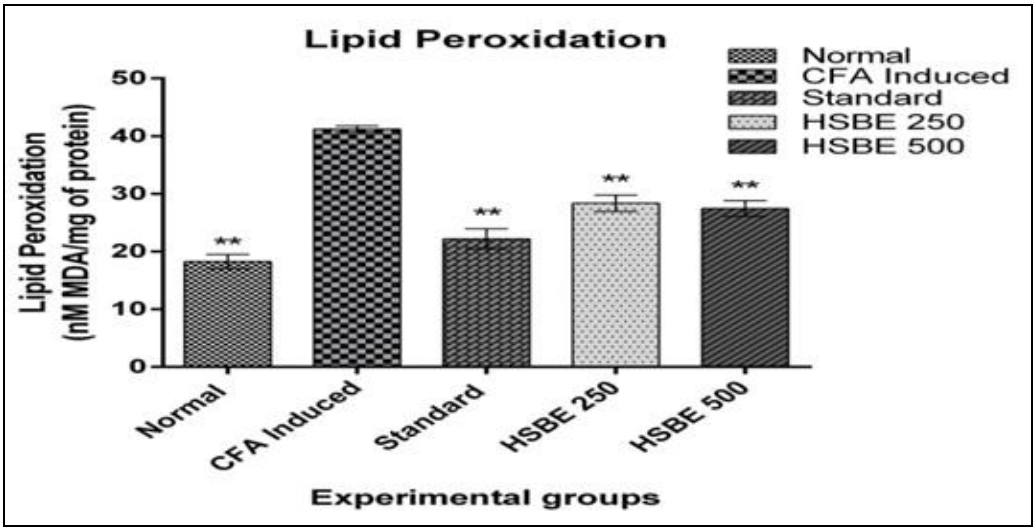

FIG. 6: EFFECT OF HSBE ON LIPID PEROXIDATION LEVELS IN CFA INDUCED DIABETIC RATS AND TREATED GROUPS. EACH COLUMN REPRESENTS THE MEAN ± S.E.M (n=6/GROUP). THE SIGNIFICANCE LEVELS ARE DENOTED BY ##P<0.01, COMPARED WITH NORMAL CONTROL (GROUP I); **P<0.01, COMPARED WITH DISEASE CONTROL.

3.8.2 Glutathion reductase:

Oxidative stress associated with CFA induced polyarthritis was evaluated by measuring level of GSH in the inflamed liver tissues. FCA injection into hind paws decreased the tissue GSH, which is naturally occurring antioxidant in body. In normal control rats tissue supernatant level changes. Both the doses of extract produces an increase in the level of GSH **Fig.7**

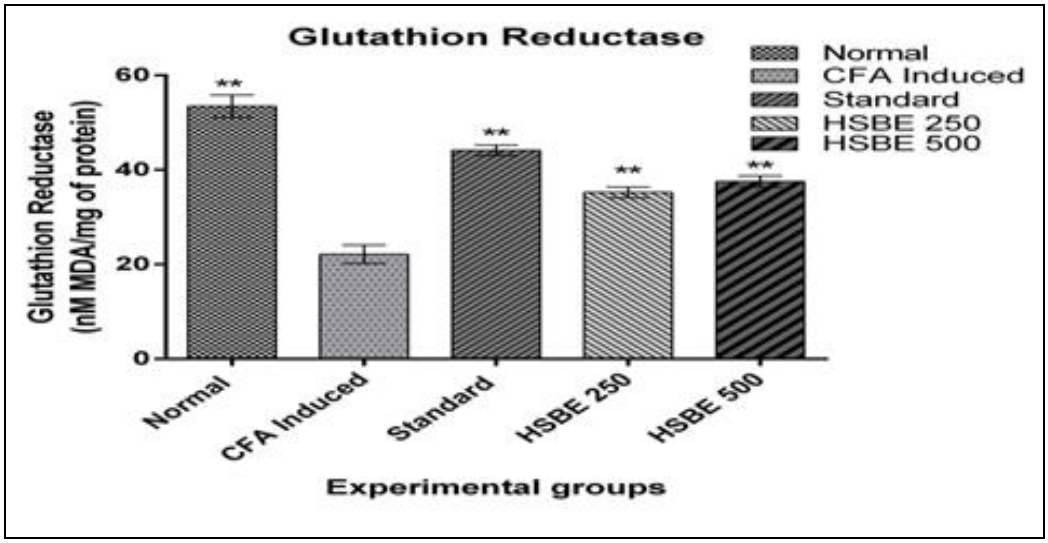

FIG.7: EFFECT OF HSBE ON GLUTATHION REDUCTASE LEVELS IN STREPTOZOTOCIN INDUCED DIABETIC RATS AND TREATED GROUPS. EACH COLUMN REPRESENTS THE MEAN ± S.E.M (n=6/GROUP). THE SIGNIFICANCE LEVELS ARE DENOTED BY ##P<0.01, COMPARED WITH NORMAL CONTROL (GROUP I); **P<0.01, COMPARED WITH DISEASE CONTROL.

3.8.3 Catalase Activity:

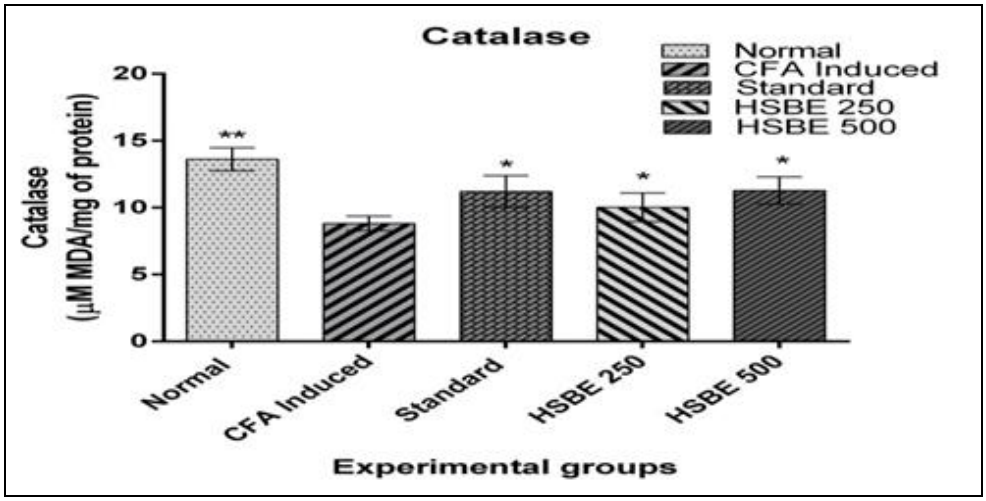

FIG. 8: EFFECT OF HSBE ON CATALASE LEVELS IN CFA INDUCED DIABETIC RATS AND TREATED GROUPS. EACH COLUMN REPRESENTS THE MEAN ± S.E.M (n=6/GROUP). THE SIGNIFICANCE LEVELS ARE DENOTED BY ##P<0.01, COMPARED WITH NORMAL CONTROL (GROUP I); *P<0.05, **P<0.01, COMPARED WITH GROUP II.

3.9 Radiological images:

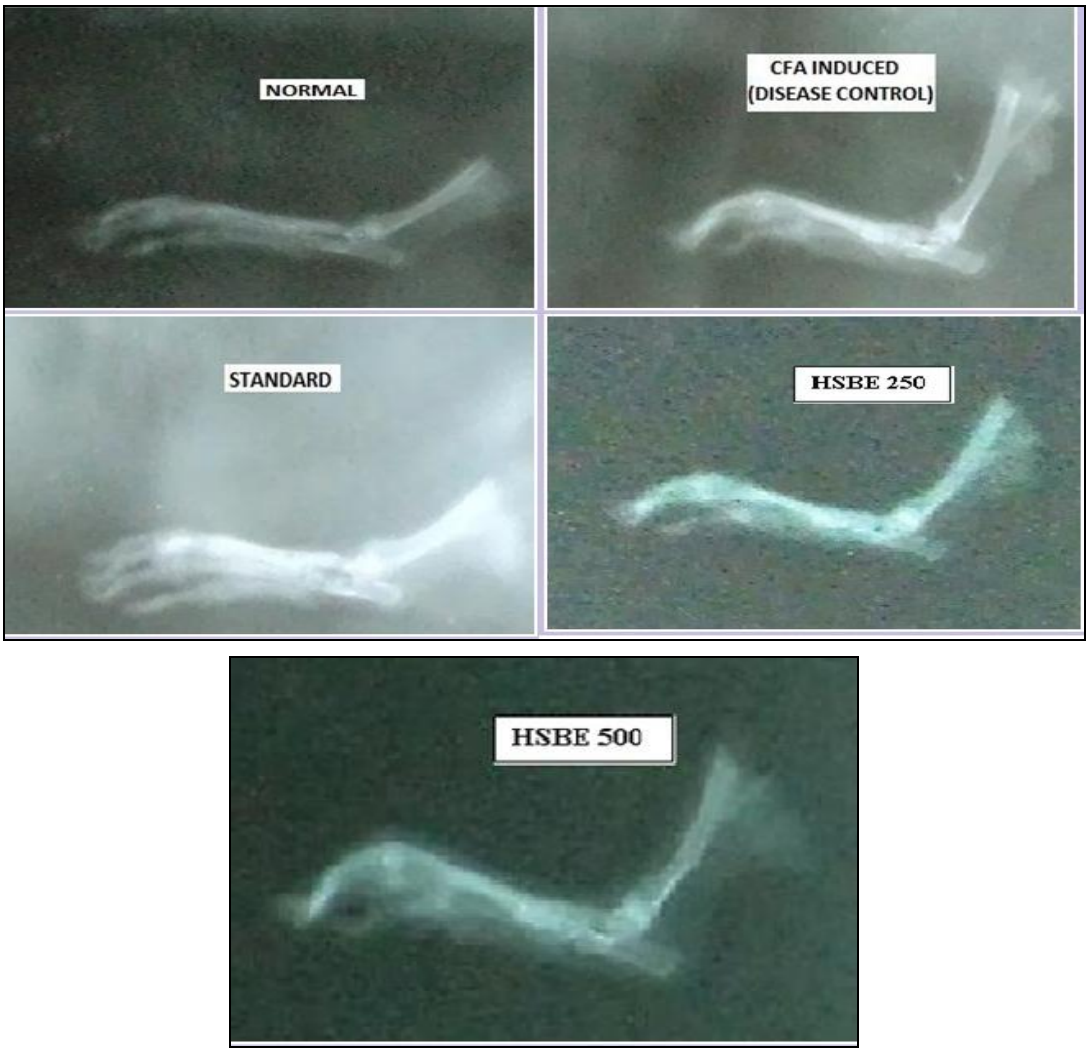

FIG. 9: RADIOGRAPHIC IMAGES SHOWING EFFECT OF DIFFERENT TREATMENTS ON TIBIOTARSAL JOINTS OF CFA INDUCED ADJUVANT ARTHRITIS RATS AND VEHICLE CONTROL RATS.

3.10 Histopathological analysis of tibiotarsal joints:

In the tibiotarsal joints of normal rats (Group I), there was presence of normal connective tissues structure with the absence of necrosis. It does not show any evidence of lymphocytic infiltration **(Fig. 10A**). The tibiotarsal joint of disease control rats (Group II) showed massive influx of inflammatory cells, synovial hyperplasia and accumulation of poly-morphonuclear cells in the joint and edema noticed. It also shows the presence of higher degree of necrosis (**Fig.10B**). Treatment with standard drug (Group III, Piroxicam 10 mg/kg, p.o.) showed normal connective tissue of tibiotarsal joint with the presence of lower degree of edema. There was absence of necrosis. **Fig.10C**). The rats treated with HSBE (250 & 500 mg/kg) showed presence of mild necrosis with low amount of poly-morphonuclear cells accumulation but mild edema was present (**Fig. 10D & Fig. 10E**).

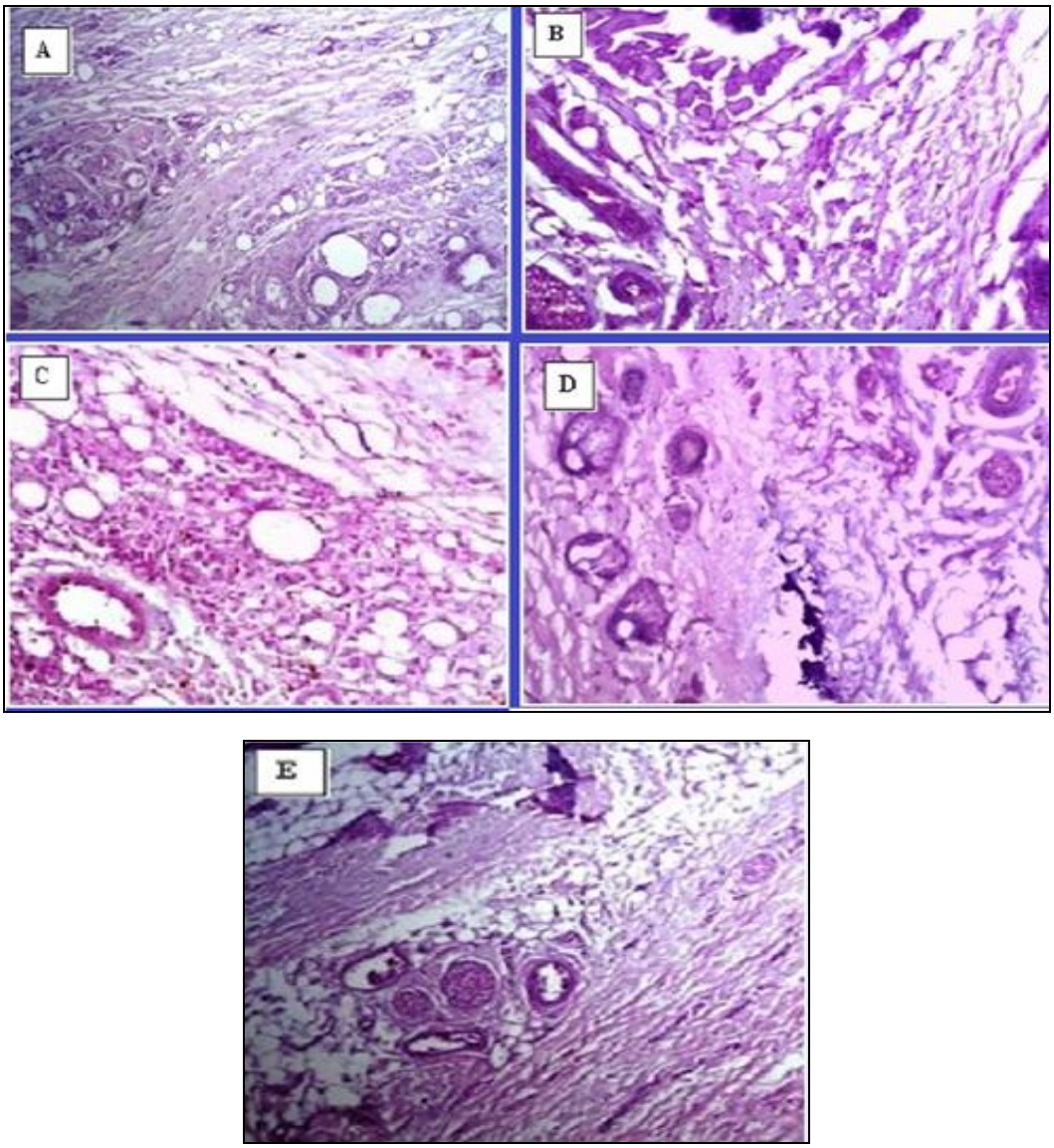

FIG. 10: HISTOPATHOLOGICAL REPRESENTATION (IMAGES X 100 MAGNIFICATION) OF TIBIOTARSAL JOINTS STAINED WITH H&E. (A) NORMAL RAT (B) CONTROL RAT (C) PIROXICAM (10 MG/KG) TREATED RAT (D & E) HSBE (250 & 500 mg/kg) TREATED RAT. ARE TYPICAL AND REPRESENTATIVE OF EACH STUDY GROUP.

DISCUSSION: The phytochemical screening of the secondary metabolites of the plant parts present of different component when successive extraction has done. The different reaction color of the experiments has denoted the intensity of the present of the compounds. The alkaloidal compounds are most present in the methanolic fractions whereas carbohydrates, flavonoids, saponins, glycosides and phenolic compounds are present in the hydro-alcoholic fractions. The alkaloids are less soluble in

the polar solvent i.e. water or ethanol but the carbohydrates, flavonoids and phenolic compounds are easily soluble in more polar solvent than low polar fraction. Although the Hydro-alcoholic mixture is high polar solvent, and some portion of alkaloid is also present in this medium may as in form of alkaloidal salts.

In the present study, rats were selected to induce arthritis because rats develop a chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid disease [28]. Changes in body weight have also been used to assess the course of the disease and the response to therapy of anti-inflammatory drugs [29]. During experimental period significant changes in body weight was observed as shown in figure 1. Body weight, food intake and metabolism are affected by immunity and inflammation and they are regulated by a cytokine-like hormone known as Leptin.

It has been previously reported that T cell proliferation to promote Th1 responses in autoimmunity is stimulated by leptin [30-31]. In CFA induced arthritis, within 24 h. of the administration of CFA the plasma leptin levels were rapidly increased which led to anorexia and loss of body weight [32]. As the severity of disease increased, the body weight also reduced significantly. On the other side it may also proposed that absorption of 14C-glucose and 14C-leucine in rat's intestine was reduced in the case of inflamed rats [33]. Treatment with anti-inflammatory drugs, the decrease in absorption was nullified and it shows that the anti-inflammatory drugs have corrected the decreased absorption capacity of intestine during inflammation [34].

During experiment, it was observed that the normal rats gain body weight, whereas arthritic rats reduce their body weight. Extract treated rats significantly improved the body weight and in standard group rats, Piroxicam shows more weight increment than extract treated rats after 7 days of treatment.

The inhibition of CFA induced inflammation (paw volume) in rats (**Fig.2**.) is an established model for evaluating anti-arthritis activity of drugs, which has been used frequently to assess anti-edematous effect of natural products [35]. The immunologically mediated complete Freund's adjuvant induced arthritic model of chronic inflammation is considered as the best available experimental model of RA [36]. Freund's Complete Adjuvant is inactivated and dried mycobacteria which are mainly responsible for stimulation of cell-mediated immunity which ultimately increased the production of certain immunoglobulins. CFA induced arthritis is a primary and secondary chronic arthritis [37].

Primary is inflammatory phase where generation of prostaglandin occurs and secondary immunological state in which autoantibodies is generated. So, administration of CFA caused joint swelling, infiltration of inflammation cells and joint cartilage erosion. Since the extract significantly inhibited paw edema, induced by CFA, this finding suggests a possible inhibition of primary phase of cyclooxygenase synthesis by the extract in dose dependent manner that link to prostaglandins. Moreover Release of certain inflammatory mediators including cytokines (IL-1B and TNF-alpha), interferon's and platelet derived growth factor (PDGF) are responsible for the initiation of pain along with swelling of the limbs and joints, bone deformations and disability of joint function [38]. The increased in the paw thickness after sub-plantar administration of CFA is reflecting the status of arthritis. The extracts decreased the thickness of paw via inhibition of release of inflammatory mediators, indicating its anti-inflammatory potential in CFA induced arthritis.

The arthritis manifestation occurs on bone joint. Inflammatory status on hind paw is the evolutionary indication of arthritis. Arthritic index (**Fig. 3**) is the average of the score given to severity of the lesions in joints [39]. In CFA induced arthritis, arthritis score is index of the joint inflammation after immunization (secondary stage of CFA induced) [40].

The HSBE extract comparatively suppressed the arthritic index as compared with disease control group animals. Prominent immunological abnormalities that may be important in pathogenesis of RA include immune complexes are

found in joint fluid cells and in vasculitis. Plasma cells produce antibodies e.g., rheumatoid factor that contribute to these complexes [41]. Higher the levels of serum rheumatoid factor (**Fig. 4**), higher are the development of inflammation. The extract and the standard drug treated animals groups showed lesser serum RF than disease control group.

The organ spleen is the reservoir for the cells and antibody production. The increased of weight of spleen associated with the overproduction of anti-body and this may be due to the immune stimulatory effect of the HSBE (**Fig.5**). CFA induced arthritis is associated with accumulation of mono and poly-morphonuclear cells in the articular joints [42]. Activation of morphonuclear cells causes degeneration of joint structure, including synovial fluid and cartilage via down regulation of endogenous anti-oxidant defense system [43]. Moreover, Oxidative stress inflicts damage to joints because of excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in arthritis [44-45].

Generation of superoxide radicals in body results in collagen degradation that may trigger other inflammatory reactions and tissue destruction through activation of neutrophils [46]. Cells contain a number of anti-oxidants such as superoxide dismutase, catalase and glutathione peroxidase which prevent the damage caused by ROS [47]. The higher level of MDA production in tissues is considered to the generation of superoxide radical. The present study shows that lower level of MDA of HSBE extract (250 mg/kg & 500 mg/kg) can suppress the ROS, that were more elevated in case of disease control group. Catalase is another anti-oxidant enzyme which protects cells from superoxide radicals by interacting endogenous $H_2O_2$and liberating water and oxygen [48]. Earlier studies have shown that catalase levels decreased in chronic inflammatory states [49]. The resultant data revealed that the HSBE may have role to inhibiting the generation of reactive oxygen species (ROS) in CFA-induced arthritic animals.

The investigation on the arthritic rats showed a soft tissue swelling around the ankle joints during the acute phase of arthritis. In our study radiographic examination of ankle joints was done (**Fig. 9**). Ankle joint X-rays of untreated arthritic rats showed excessive soft tissue swelling, and bone erosion. In case of disease control, the bones are unprotected by cartilage, since they are directly exposed to proteolytic enzymes such as collagenases, glycohydrolases and neutral proteases degrading the cartilage. As a result, the pannus invades the joint and sub-chondral bones and eventually the joint is destroyed and undergoes fibrous fusion or ankylosis [50]. The extract fractions of HSBE and the standard drug piroxicam showed reduced soft tissue swelling and bone erosion in X-rays of ankle joint. This finding confirms that HSBE can effectively restore the disease progression in arthritic cases.

Bone joints are the initial sites of the inflammatory process of RA. The histological changes in ankle joints of disease control shows congestion of vessels and presence of inflammatory cells, hyperplasia, and accumulation of abundant mono-morphonuclear and poly-morphonuclear cells in the joint space (**Fig. 10**). The mono & poly morphonuclear cells, lymphocytes, monocytes and macrophages produce inflammatory cytokines. Therefore, the inhibition of leucocytes mitigation to the inflammatory joint space may cause the reason for protect the joint erosion. The plant extract shows little joint erosion and inflammatory cells migration. This may due to inhibitory effect of mono & poly-morphonuclear cells in the joint space of RA group.

Since our investigations have shown that HSBE possess significant anti-arthritic activity in CFA induced experimental animals as evidenced by both signs and pathology scores. The further studies also be require to fully elucidate the anti-RA constituents and mechanisms of action.

The histopathological study of the disease control rats showed massive influx of inflammatory cells, synovial hyperplasia and accumulation of poly-morphonuclear cells in the joint and edema noticed. Whereas the HSBE can improved the cell structure and the mechanism is still unknown and have the significant aspect to investigate the cell molecular mechanism

CONCLUSION: Natural products research remains one of the main means of discovering bioactive compounds. The studies concerning the impact of ethnopharmacological significance of the medicinal plants. The bark extract of *P. rubra* plant has prepared as the villagers use to recover their pain. The experimental outcome proves the HSBE has the properties to check the arthritic pain. In this investigation, we observed from the pharmacological and biochemical parameters that the HSBE is a significant anti-inflammatory agent in the treatment of rheumatoid arthritis. Hence, in future it is necessary to identification the lead active compounds and evaluation its anti-arthritis activities on human clinical trials.

CONFLICT OF INTEREST: The authors' have no conflict of interests.

ACKNOWLEDGEMENTS: The authors are grateful to Dr. B. B. Kakoti (Associate professor, Dept. of Pharmaceutical sciences, Dibrugarh University, India) for his kind help to arrange the CFA and give permission to use his laboratory for this experiment. We are also thankful to Ms.Babita kumari and Mr. Bhaben Gogoi (laboratory technical assistance, Pharmacognosy research laboratory, Dept. of Pharmaceutical sciences, Dibrugarh University, India) for their kind co-operation during animal care, handling and experimental procedure.

REFERENCES:

1. D. M. Meyer, M. I. Jesson, X. Li, M. M. Elrick, C. L. F. Shippy, J. D. Warner, C. J. Gross, M. E. Dowty, S. K. Ramaiah, J. L. Hirsch, M. J. Saabye, J. L. Barks, N. Kishore, D. L. Morris, "Anti-inflammatory activity and neutrophil reductions mediated by the JAK1/JAK3 inhibitor, CP-690,550 in rat adjuvant-induced arthritis," Journal of Inflammation, vol. 7, pp. 41, 2010.
2. J. S. Bang, D. H. Oh, H. M. Choi et al., "Anti-inflammatory and antiarthritic effects of piperine in human interleukin 1β-stimulated fibroblast-like synoviocytes and in rat arthritis models," Arthritis Research & Therapy, vol. 11, no. 2, pp. 9, 2009.
3. I. B. Mcinnes and G. Schett, "The Pathogenesis of Rheumatoid Arthritis," The New England Journal of Medicine, vol. 365, pp. 2205-2219, 2011.
4. M. Mijiyawa, "Epidemiology and semiology of rheumatoid arthritis in Third World countries," Revue du rhumatisme English ed, vol. 62, no. 2, pp. 121-226, 1995.
5. I. A. V. D. Oever, A. M. V. Sijl, T. Michael, "Nurmohamed. Management of cardiovascular risk in patients with rheumatoid arthritis: evidence and expert opinion," Therapeutic Advances in Musculoskeletal Disease, vol. 5, no. 4, pp. 166–181, 2013.
6. T. Doan, E. Massarotti, "Rheumatoid Arthritis: An Overview of New and Emerging Therapies," The Journal of Clinical Pharmacology, vol. 45, no. 7, pp. 751–762, 2005.
7. C. J. Zhenga, X. X. Zhaoa, H. W. Aia, B. Lina, T. Hana,Y. P. Jianga, X. Xingb, L. P. Qina, "Therapeutic effects of standardized Vitex negundo seeds extract on complete Freund's adjuvant induced arthritis in rats," Phytomedicine, vol. 21`, no. 6, pp. 838-846, 2014.
8. M. S. Bansod, V. G. Kagathara, R. R. Pujari, V. B. Patel, H. H. Ardeshna."Therapeutic effect of a poly-herbal preparation on adjuvant induced arthritis in wistar rats," International Journal of Pharmacy and Pharmaceutical Sciences, vol. 3, no. 2, pp. 186-192, 2011.
9. S. Ahmed, J. Anuutiyo, C. J. Malemud, T. M. Haqqi, "Biological basis for the use of botanicals in osteoarthritis and rheumatoid arthritis: a review," Evidence-Based Complementary and Alternative Medicine, vol. 2, pp. 301-308, 2005.
10. A. T. Thite, R. Patil, S. R. Naik. "Anti-arthritic activity profile of methanolic extract of Ficus bengalensis: Comparison with some clinically effective drugs," Biomedicine & Aging Pathology, vol. 3, no. 4, pp. 207-217, 2014.
11. C. koli, P. A. Akah, S. Nwafor. "Anti-inflammatory activity of plants," Journal of Natural Remedies, vol. 3, no. 1, pp. 1-30, 2003.
12. G. Kaur, D. Patel, M. G. Sawant, P. Deshmukh. "Herbal Medicine A natural cure to arthritis. Indian Journal of Natural Products and Resources, vol.4, no.1, pp. 27-35, 2013.
13. S. Singh, D. K. Majumder, "Effect of fixed oil of Ocimum sanctum against experimentally induced arthritis and joint edema in laboratory animals," Pharmaceutical Biology, vol. 34, no. 3, pp. 218-22, 1996.
14. S. G. Joshi, "Medicinal Plants," Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi, pp 46, 2000.
15. S. Ekambaram, S. S. Perumal, V. Subramanian, "Evaluation of anti-arthritic activity of Strychnos potatorum Linn seeds in Freund′s adjuvant induced arthritic rat model," BMC Complementary and Alternative Medicine, vol. 10, pp. 1–9, 2010.
16. A. Mehta, N. Sethiya, C. Mehta, G. B. Shah, "Anti-arthritis activity of roots of Hemides musindicus R.Br. (Anantmul) in rats," Asian Pacific Journal of Tropical Medicine, vol. 5, no. 2, pp. 130-135, 2012.
17. T. Manaharana, S. Chakravarthib, A. K. Radhakrishnanc, U. D. Palanisamy, "In vivo toxicity evaluation of a standardized extract of Syzygium aqueum leaf, Toxicology Reports," vol. 1, pp. 718–725, 2014.
18. D. L. Quan, G. M. Thiele, J. Tian, D. Wang, "The development of novel therapies for rheumatoid arthritis," Expert Opinion on Therapeutic Patents,vol. 18, pp. 723-738, 2008.
19. J. Paval, S. K.Kaitheri, B. K. Potu, S. Govindan, R. S. Kumar, S.N. Narayanan. "Anti-arthritic potential of the plant Justicia gendarussa Burm F," Clinics, vol. 64, no. 4, pp. 357-62, 2009.
20. C. K. Cain, J. M. Francis, M. A. Plone, D. F. Emerich, M. D. Lindner, "Pain- related disability and effects of chronic morphine in the adjuvant induced arthritis model of chronic pain," Physiology & Behavior, vol. 62, no. 1, pp. 199–205, 1997.
21. Y. F Chen, P. Jobanputra, P. Barton, S. Bryan, A. Fry-Smith, G. Harris , R. S. Taylor, "Cyclooxygenase-2 selective non-steroidal anti-inflammatory drugs (etodolac, meloxicam, celecoxib, rofecoxib, etoricoxib, valdecoxib

and lumiracoxib) for osteoarthritis and rheumatoid arthritis: A systematic review and economic evaluation," Health Technology Assessment, vol. 12, no. 11, pp. 1–278, 2008.

22. L. A. B. Joosten, E. Lubberts, M. M. A. Helsen, V. D. Berg, "Amelioration of established murine collagen induced arthritis with anti-IL-1 treatment," The Journal of Immunology, vol. 95, pp. 237-243, 1994.
23. R. Yazdanparast, A. Ardestani, S. Jamshidi, "Experimental diabetes treated with Achillea santolina: effect on pancreatic oxidative parameters," Journals of Ethnopharmacology, vol. 112, pp. 13-18, 2007.
24. K. A. Sinha,"Colorimetric assay of catalase," Analytical Biochemistry , vol. 47, pp. 389-394, 1972.
25. G. L. Elman, "Tissue sulfhydryl groups," Archives of biochemistry and biophysics, vol. 82, pp. 70-79, 1959.
26. L. A. B. Joosten, M. M. A. Helsen, V. D. F. A. J. Loo, V. D. W.B. Berg. "Role of cytokines in rheumatoid arthritis," Arthritis & Rheumatology, vol. 39, pp. 797–809, 1996.
27. J. R Connor, P. T Manning, S. L Settle, W. M Moore, G. M Jerome, R. K Webber, F. S Tjoeng, M. G. Currie. "Suppression of adjuvant-induced arthritis by selective inhibition of inducible nitric oxide synthase," European Journal of Pharmacology, vol. 273, pp. 15–24, 1995.
28. G. B. Eric, J. L. Lawrence, "Rheumatoid arthritis and its therapy," Williams and Wilkins Company, altimore, PP. 579- 95, 1996.
29. C. V. Winder, L. A. Lembke, M. D. Stephens, "Comparative bioassay of drugs in adjuvant induced arthritis in rats: flufenamic acid, mefenamic acid and phenylbutazone," Arthritis & Rheumatology, vol. 12, no. 5, pp. 472-482, 2005.
30. G. Matarese, D. A. Giacomo, V. Sanna, G. M. Lord, J. K. Howard, D. A. Tuuro, "Requirement for leptin in the induction and progression of autoimmune encephalomyelitis," The Journal of Immunology, vol. 166, no. 10, pp. 5909–16, 2001.
31. M. Vishwanath, K. Patil, A. D. Kandhareb, S. D. Bhisea, "Anti-arthritic and anti-inflammatory activity of Xanthium srtumarium L. ethanolic extract in Freund's complete adjuvant induced arthritis," Biomedicine & Aging Pathology, vol. 2, pp. 6–15, 2012.
32. M. Barbier, C. Cherbut, A. C. Aube, H. M. Blottiere, J. P. Galmiche, "Elevated plasma leptin concentrations in early stages of experimental intestinal inflammation in rats," Gut, vol. 43, no. 6, pp. 783–90, 1998.
33. S. Somasundaram, J. Sadique, A. Subramoniam , "In vitro absorption of [14C] leucine during inflammation and the effect of anti-inflammatory drugs in the jejunum of rats," Biochemical Medicine, vol. 29, pp. 259–264, 1983.
34. S. Somasundaram, J. Sadique, A. Subramoniam , "Influence of extra-intestinal inflammation on the in vitro absorption of 14C-glucose and the effects of anti-inflammatory drugs in the jejunum of rats," Clinical and Experimental Pharmacology and Physiology, vol. 10, no. 2, pp. 147–152, 1983.
35. I. Igbe, F. P. Ching, A. Eromon." Anti-inflammatory activity of aqueous fruit pulp extract of Hunteria umbellata k. schum in acute and chronic inflammation," Acta Poloniae Pharmaceutical and Drug Research, vol. 67, no. 1, pp. 81-85, 2010.
36. W. B. V. D. Berg, L. A. B. Joosten, M. M. A. Helsen, F. A. J. V. D. Loo, "Amelioration of established murine collagen-induced arthritis with anti-IL-1 treatment," Clinical & Experimental Immunology, vol. 95, pp. 237–243, 1994.
37. D. T. Walz, M. J. Dimartino, A. Misher, "Adjuvant induced arthritis in rats. II. Drug effects on physiologic, biochemical and immunologic parameters," Journal of Pharmacology and Experimental Therapeutics, vol. 178, no. 1, pp. 223-231, 1971.
38. G. B. Eric, J. L. Lawrence, "Rheumatoid arthritis and its therapy, The Textbook of therapeutic drugs and disease management," Williams and Wilkins Company, Baltimore, ed. 6, pp. 579-595, 1996.
39. F. C. Colpaert. "Evidence that adjuvant arthritis in the rat is associated with chronic pain," Pain, vol. 28, pp. 201-222, 1987.
40. Y. Yu, Z. Xiong, Y. Lv, Y. Qian, S. Jiang, Y. Tian. "In vivo evaluation of early disease progression by X-ray phase-contrast imaging in the adjuvant-induced arthritic rat," Skeletal Radiology, vol. 35, no. 3, pp. 156–64, 2006.
41. N. Pathak, P. Gohil, N. B. Patel, S. Kasture, N. Jivan, Y. Bhalodia. "Curative Effect of Albizia lebbeck Methanolic Extract against Adjuvant Arthritis-With Special Reference to Bone Erosion,"International Journal of Pharmaceutical Sciences and Drug Research, vol. 1, no. 3, pp.183-187, 2009.
42. A. V. Borght, P. Geusens, J. Raus, P. Stinissen, "The autoimmune pathogenesis of rheumatoid arthritis: role of autoreactive T cells and new immunotherapies," Seminars in Arthritis and Rheumatism, vol. 31, pp. 160–75, 2001.
43. G. Li , Y. Liu, N. S. Tzeng , G. Cui, M. L. Block, B. Wilson, L. Qin, T. Wang, B. Liu, J. Liu, J. S. Hong, "Protective effect of dextromethorphan against endotoxic shock in mice," Biochemical Pharmacology, vol.69, pp. 233–240, 2005.
44. A. Mirshafiey, M. Mohsenzadegan, "The role of reactive oxygen species in immunopathogenesis of rheumatoid arthritis," Iranian Journal of Allergy, Asthma and Immunology, vol. 7, no. 4, pp. 195–202, 2008.
45. D. C. Phillips, H. K. Dias, G. D. Kitas, H. R. Griffiths, "Aberrant reactive oxygen and nitrogen species generation in rheumatoid arthritis (RA): causes and consequences for immune function, cell survival, and therapeutic intervention," Antioxidants & Redox Signaling, vol. 12, pp. 743–85, 2010.
46. D. D. Bandawane, S. Beautikumari, S. S. Gate, A. N. Patel. "Evaluation of anti-arthritic activity of ethyl acetate fraction of Cassia auriculata Linn," leaves,"Biomedicine & Aging Pathology, vol. 4, no. 2, pp. 105–115, 2014.
47. C. M. Weydert, J. J. Cullen,"Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue," Nature Protocols, vol. 5, pp. 51-66. 2009.
48. S. Sharma, D. Sahu , H. R. Das, D. Sharma, "Amelioration of collagen-induced arthritis by Salix nigra bark extract via suppression of pro-inflammatory cytokines and oxidative stress," Food and Chemical Toxicology, vol, 49, pp. 3395–406, 2011.
49. U. R. Ijeoma, N. C. Onyebuchi, U. S. Ositadimma, "Evaluation of the anti-arthritic effect of Sterculia tragacantha. (lindl.) Leaf extract in rats," American Journal of Pharmacology and Toxicology2014; 9 (2): 107-113.
50. M. S. Bansod,V. G. Kagathara, R. R. Pujari, V. B. Patel, H. Ardeshna. "Therapeutic effect of apoly-herbal preparation on adjuvant induced arthritis in wistar rats," International Journal of Pharmacy and Pharmaceutical Sciences, vol. 3, no. 2, pp. 186-192, 2011.

How to cite this article:
Mondal P, Das S, Mahato K, Borah S, Junejo JA and Zaman K: Evaluation of Anti-Arthritic Potential of the Hydro-Alcoholic Extract of the Stem Bark of *Plumeria Rubra* in Freund's Complete Adjuvant-Induced Arthritis in Rats.. Int J Pharm Sci Res 2016; 7(9): 3675-88.doi: 10.13040/IJPSR.0975-8232.7(9).3675-88.