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Amelioration of experimental rheumatoid arthritis by selected ultra-diluted preparations by down regulating increased expression of TNF-α & IL-6

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The current work explored the inhibitory effect of selected homeopathic drugs, in experimental models of inflammation and CFA-induced arthritis. Twelve groups of animals were made, each containing 6 animals. The selected homeopathic drugs (causticum, calcarea, medorrhinum, mercurius, formica, proteus, silica, sulphur, thuja), placebo and standard drug, Indomethacin. In CFA model, treatment groups and the reference drug were administered daily for a period of 21 days. Dysfunction in joints was evaluated by parameters such as joint diameter, expression of inflammatory markers (TNF-α, IL-6). Findings of the study revealed that on CFA administration, there is a significant ($p<0.01$) increase in joint diameter in all the tested animals. On day 3, we found highest increase in the joint diameter in all treatment groups. Medorrhinum, silica, sulphur showed significant ($p<0.01$) decrease in joint diameter on day 21. Significant ($p<0.05$) reduction in paw edema was observed at 5 h post carrageenan administration. IHC of NF-kB in CFA treated group revealed presence of vacuoles, infiltration of inflammatory cells. However, prominent reversal of joint damage was seen in homeopathic drugs (medorrhinum, silica, sulphur) and indomethacin. Study inferred that the homeopathic drugs (medorrhinum, silica, sulphur) and indomethacin were found to be potent in ameliorating inflammation.

Keywords: Histopathology, Homeopathic drugs, Indomethacin, Inflammation, Medorrhinum, Rheumatoid arthritis
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Rheumatoid arthritis (RA) is an autoimmune disorder which causes tenderness. It manifests as progressive disability, other systemic complications and can cause death. The disease has progressive nature, which causes inflammation in joints, synovial proliferation, and can cause destruction of articular cartilage[1]. The symptoms are painful and swollen joints. Wrist and hand joints are affected the most. The primary target of RA is synovial joints. Excessive fluid is produced in the joints, cartilage is destructed, and erosion of marginal bones, ligaments and tendons are stretched and damaged due to proliferation of synovial joints[2]. Also, there are other clinical features associated with the disease such as low red blood cell count, inflammation around the heart and lungs. In RA, the systemic reaction of individual to its self-epitope results in trigger of inflammation in body, particularly in the synovium. The precise etiology is unknown but though to cause or triggered by variety of factors including genetic, environmental, lifestyle or other external factors involved[3]. RA is multifaceted process that involves proliferation of synovial cells, pannus formation, fibrosis, erosion of bones and cartilages. It is mediated through inter-reliant network of prostanoids, proteolytic enzymes and cytokines and among all, pro-inflammatory cytokines IL-1 (Interleukin-1), TNF-α (Tumor Necrosis Factor-alpha) which are central mediators of RA. IL-1 has direct co-relation with the disease activity. It has been seen that the patients with massive RA has high number of synovial cells and presence of higher amount of circulating IL-1. IL-6 is another pro-inflammatory cytokine exhibits involvement in immunity, bone metabolism, inflammation and some endocrine functions.

Production of IL-6 is initiated by number of different cells include monocytes, lymphocytes, endothelial cells and fibroblasts. Thousands of patients having inflammatory disorders suffer from gastrointestinal disturbances due to long-term consumption of NSAIDs (Non-Steroidal Anti-Inflammatory Drugs)[4] and also taking into account the

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chronic nature of these disorders, compliance became another issue associated with these expensive and fatal drugs. Although a high number of patients suffering from inflammatory disorders relies on supportive and replacement drugs either used solo or in fusion with traditional therapy for enhanced results[3]. So, discovering new compounds of natural origin in homeopathic system of medicine with potential therapeutic action and emblematic adverse effects have been practiced vigorously[5]. Medicinal plants are being used either in the form of homeopathic drugs in the prevention and management of inflammatory disorders in prophylactic way.

Homeopathic drugs (causticum, calcarea, medorrhinum (medorrhium), mercurius, formica, proteus, silica, sulphur, thuja) are available in different formulations or preparations for the treatment of different ailments. These selected homeopathic drugs have been studied in some depth and several compounds with varying effects identified. Homeopathic causticum is a popular remedy made from the compound called potassium hydrate. As a homeopathic remedy, causticum has many immense health benefits. It is a key remedy for joint and arthritic conditions and skin problems like severe burns. Homeopaths also prescribe it for tremors and paralysis, urinary disorders, respiratory disorders, hemorrhoids, fibromyalgia and more[6]. Calcarea carbonica is obtained from the calcium in oyster shell. It is commonly used for different types of pain including bone and joint pain, arthritis, bone growth. It is also used for anxiety, phobias and disturbances in menstruation, imbalance in hormones and PMS[7]. The medorrhinum homeopathic remedy is produced from *Neisseria gonorrhoeae* bacterium, causative agent for gonorrhea. It is also prescribed for spine, kidneys, nerves and for mucous membranes[8]. Mercurium corrosives is homeopathic remedy which is prepared from mercuric chloride and employed in treating different types of ulcers, primarily inflammatory bowel disease, ulcerative colitis[9]. Formica rufa is a homeopathic remedy made from the red fire ant. To make formica rufa, the fire ant's body is ground up and diluted with lactose powder, alcohol, or distilled water. The process of dilution continues until there is no cellular evidence of the ant in the final product. The different diluted products are made into pellets, a tincture, or a water-based liquid. It may also be given to relieve the symptoms associated with gout, rheumatoid arthritis, vertigo, nausea and headache[10]. Silica is non-metallic element widely present in earth's crust and is an important element for plants. In humans, the silicon strengthens teeth, hairs and nails and present in connective tissue. Homeopathic silica is also used for eye, nose, throat, bone, joint, nerve system, and digestive problems. Silica is especially useful for arthritis, knee inflammation of the bone, weak wrists and subcutaneous nodules of hip joints when there is painful swelling. There are several nervous system problems where homeopathic silica can be useful. This can include a migraine headache where the person has disturbed vision, dizziness and a sharp pain that affects the head[11]. It also helps other nervous system problems, including tremors or paralysis, neuralgia, vertigo, and coldness of the extremities. Sulphur is an elementary substance, occurs in nature in the form of brittle crystalline solid, it burns with a blue flame in air. The mineral sulphur is present in every cell of the body, especially those present in the hair, skin and nails. It is an extremely important homeopathic remedy. It is used for conjunctivitis, eczema, cold, digestive disorders, nausea, constipation, hemorrhoids, diarrhea and shortness of breath[12]. *Thuja occidentalis* belongs to the family Cupressaceae. It is extensively cultivated as an ornamental plant and is native to Eastern Canada, Central and Upper Northeastern United States. It is used in the treatment of respiration related conditions like bronchitis, cold sores and bacterial skin infections. It is used to treat a wide variety of painful conditions such as osteoarthritis, nerve disorders and trigeminal neuralgia. The oil of thuja is applied over warts, cancer, skin diseases, insect repellent and alleviates joint and muscle pain[13]. There are very few studies that have targeted the eventual benefice of these selected homeopathic drugs in attenuation of inflammation and arthritis. The present study showing the *in-vivo* effects of selected homeopathic drugs on reduction of joint dysfunction and inflammation in investigational framework of rheumatoid arthritis.

Materials and Methods

Investigational animals

Wistar rats having weight between 150-180 g were acquired from the Central Animal Facility of AIIMS. The rats were kept in normal laboratory conditions provided 12 h light-dark cycle and regulated

temperature (20-25°C) and humidity. The rats become accustomed to the environment for a week prior to experimentation with free access to water and normal dietary regime. Experimental rats were grouped within 6. Homeopathic medicines were given by oral route, using feeding cannula, and were observed for incidence of mortality and sign of intoxication daily. Animals were housed in labeled cages in groups of six and their fur was tagged with methylene blue for identification. The protocol was approved by the Institutional Animal Ethics Committee (983/IAEC/16). After acceptance, animals were taken, weighed and disseminate arbitrarily into appropriate groups for performing the study. The experiments were carried out in the premises of the Animal House, Department of Pharmacology, AIIMS. All animal experiments were complied with the ARRIVE guidelines.

Chemical and drugs

Complete Freund's adjuvant (CFA) for inducing arthritis was purchased from Difco Laboratories Inc., USA. Reference drug, Indomethacin was procured from Sigma Chemical Co., USA. TNF-α ELISA (Enzyme-linked immunosorbent assay) kit was purchased from Diclone SAS, France. Experimental kit used for immunohistochemistry was procured from Vector labs, CA, USA. Primary antibody of IL-1, IL-6, NF-κB (Nuclear Factor-kappa B) was brought from Santa Cruz, CA, USA. Rests of the chemicals used were of analytical grade.

Test drug

Selected homeopathic drugs (causticum, calcarea, medorrhinum (medorrhium), mercurius, formica, proteus, silica, sulphur, thuja) were provided by CCRH, Ministry of AYUSH, Govt. of India, New Delhi.

Dose calculation of study drugs for experimental animals

Doses of homeopathic drugs were calculated as per advice of CCRH.

Route of administration = per oral

Vehicle for administration = De-ionized water

Standard dose of 20 uL/100 g body weight of rat considered using a micropipette and solution was mixed with 450 uL of distilled water administered orally with the help of oral catheter and flushed with 500 uL of distilled water.

Experimental design

Twelve groups of rats (n=6) were employed in the investigation which are kept on fasting overnight with access to water *ad libitum*.

Group 1: Control (normal saline 1 mL/kg/day; p.o. daily and 1 mL CFA (Difco: 0.05% (w/v) of *Mycobacterium butyricum* in mineral oil) given at subplanter region.

Group 2: Causticum was administered to rats daily and on day 0, in the left hind paw 0.1 mL CFA was given at subplantar region.

Group 3: Calcarea was administered to rats daily and on day 0, in the left hind paw 0.1 mL CFA was given at subplantar region.

Group 4: Medorrhinum was administered to rats daily and on day 0, in the left hind paw 0.1 mL CFA was given at subplantar region.

Group 5: Mercurius was administered to rats daily and on day 0, in the left hind paw 0.1 mL CFA was given at subplantar region.

Group 6: Formica was administered to rats daily and on day 0, in the left hind paw 0.1 mL CFA was given at subplantar region.

Group 7: Proteus was administered to rats daily and on day 0, in the left hind paw 0.1 mL CFA was given at subplantar region.

Group 8: Silica was administered to rats daily and on day 0, in the left hind paw 0.1 mL CFA was given at subplantar region.

Group 9: Placebo was administered to rats daily and on day 0, in the left hind paw 0.1 mL CFA was given at subplantar region.

Group 10: Sulphur was administered to rats daily and on day 0, in the left hind paw 0.1 mL CFA was given at subplantar region.

Group 11: Thuja was administered to rats daily and on day 0, in the left hind paw 0.1 mL CFA was given at subplantar region.

Group 12: Indomethacin was administered to rats daily and on day 0, in the left hind paw 0.1 mL CFA was given at subplantar region.

Carrageenan induced paw edema

The study initiates by segregating the rats into groups having 6 rats in each. Animals were left on fasting overnight, the day before initiation of the experiment. On day 0, baseline volume of paw was calculated by making use of plethysmometer and afterwards drug/vehicle was administered orally to the animals. Paw edema was initiated by 1% carrageen an injection in sub plantar region of left hind paw of animals. Readings were recorded at 1, 3 and 5 h interval after carrageenan injection to give an insight into the increase in paw volume. The percentage of edema inhibition was noted down via calculating

mean of control and treated animals using below mentioned equation:

% edema inhibition = $(V_c – V_t/V_c)$ X 100

Where, V_t is the mean of increased paw volume of rats which are treated with the test compounds and V_c is the mean of increased paw volume of the rats included in the control group[5].

Complete Freund's adjuvant induced arthritis

Wistar albino rats (n=6) were used as the experimental animals for the study. The paw joint size was determined using micrometer screw gauge. One group received the standard drug indomethacin (3 mg/kg) and other groups each received the test drugs in geometrically progressive doses. After one day pretreatment and baseline joint diameter recording, arthritis was induced by a single injection of 0.1 mL of Complete Freund's Adjuvant (CFA: 0.05%w/v *Mycobacterium butyricum* in mineral oil) in the subplantar region of the left hind paw of the rat[2,14]. The day of administration of CFA was designated as Day 1. The animals were maintained for 21 days with daily administration of vehicle, indomethacin and the test drugs in the respective doses. Joint size was being measured on days 1, 3, 7, 14 and 21. Blood was withdrawn on day 21 for estimation of circulating IL-1β, IL-6, and TNF-α level by dot blot and ELISA. Hind limbs were collected for histopathological examination. The following parameters were used to evaluate the anti-arthritic activity of the test compound:

1. Augment in joint diameter: Elevation in the joint diameter was determined by making use of standard micrometer screw gauge.
2. Joint degeneration: The extent of degeneration was estimated by histological assessment of the joint.
3. Estimation of secretory cytokine TNF-α, IL-1β, IL-6 & IL-10 level into blood stream was done by dot blot.

Determination of elevation in joint diameter in CFA model

With the help of micrometer screw gauge the paw joints of all the rats were computed and diameter of the joints was demonstrated in terms of mm. In transverse direction the screw gauge was positioned on the left paw joint of the rats and the joint diameter was noted down on day 0 before CFA injection and afterwards on 3rd, 7th, 14thand 21st day of our investigation. The elevation in the joint diameter was calculated by deducting the diameter of day 0 from the diameter of 3rd, 7th, 14thand 21st day [14].

Assessment of serum TNF-α level by ELISA

Assessment of serum TNF-α level was done into an automated ELISA analyzer by making use of ELISA kit (U-CyTech biosciences, Netherlands). The investigation was carried out according to the specification guide of the equipment provided by the maker.

Immunohistochemical analysis

To carry out the procedure of immunohistochemistry, the ankle joints of left hind paws were primarily decalcified (10% EDTA (Ethylene diamine, pH 7.4) and later preserved segments (6 µm) were made. Fixation of frozen segments were done in acetone and immunohisto chemical evaluation of NF-κB expression was done by the use of anti-rat antibodies (Santa Cruz, CA, USA) and an avidin–biotin based detection kit (Vector Labs, CA, USA). The expression of NF-κB receptor was anticipated by recording occurrence of color by making use of DAB (diaminobenzidine) substrate kit (Vector Labs, CA, USA).

Dot blot procedure for quantification of serum cytokines

Serum was diluted (3 µL) with 7 µL of PBS (phosphate buffer saline) and blotted on to nitrocellulose membrane by the use of a micropipette. Nonspecific sites were obstructed by using 5% non-fat milk. Identification of cytokine was done by making use of cytokine specific primary antibodies of IL-1 and IL-6 (Santa Cruz Biotech Inc) and HRP (Horseradish peroxidase) conjugated secondary antibodies, followed by progression with nickel-enhanced diamino- benzidine (DAB) substrate (Vector Laboratories, USA). Alpha imager EC Gel Doc system was used to capture the images and the expression of proteins was quantified in terms of percentage integrated density value (% IDV) by using Alpha View Imaging software.

Statistical evaluation

All the recorded data of the present study were expressed as Mean±SEM (n=6). ANOVA (Analysis of variance) was employed to compute the comparison within the groups with posthoc test used was Dunnett's multiple comparison tests. The statistical tools were carried out by the Graphpad Prism version 5.03, San Diego, CA, USA, where $p<0.05$ was found to be statistical significant.

Results

Effect of homeopathic drugs on carrageenan induced inflammation

The association of inflammation was quantified by carrageenan-induced paw edema assay. The different

homeopathic drugs were tested and we found that oral intake of carrageenan produced a significant ($p<0.05$) elevation in paw edema that was persistent during the study duration. Highest paw edema was investigated at 5 h post-carrageenan injection in all animals (Fig. 1). Imperative depletion in paw edema was noticed inanimals treated with indomethacin at 3 h and 5 h post-carrageenan administration. Medorrhinum, silica, sulphur showed potent anti-inflammatory activity.

Effect of homeopathic drugs on CFA-induced arthritis

CFA is injected in left hind paw at subplantar region, showed enhancement in swelling of joints, erythema and joint dysfunction in all the investigational animals. After immunization with CFA on day 14, the first indications of evolution of arthritis were visible (Fig. 2A). We observed significant elevation ($p<0.001$) in the diameter of joints of CFA-injected rats because of noticeable swelling in ankle joints in the entire experimental set

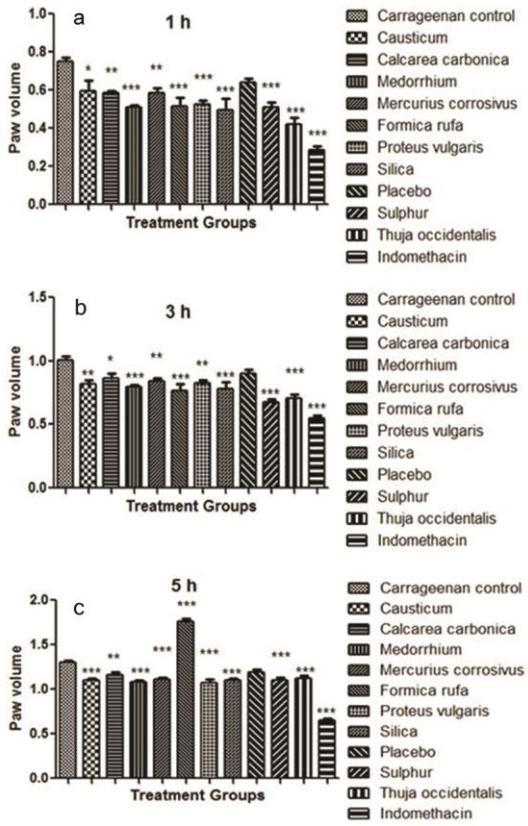


Fig. 1 — Effect of administering different homeopathic drugs oncarrageenan-induced rat paw edema model at hour 1, 3 and 5. All values are Mean±SEM (n=6). Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. *$p<0.05$, **$p<0.01$ as compared with disease control.

of animals in contrast to normal control group. Highest augmentation in joint diameter was seen on 3rd day, after that a moderate depletion in joint inflammation was observed in treated groups except the CFA-control group. After 14th day, there was minor elevation in ankle joint diameter of the CFA-control group caused by the existence of augmented immune response. Remarkable retardation ($p<0.01$) in joint swelling was noted in medorrhinum, silica, sulphur treated groups (Fig. 2B).

Effect of homeopathic drugs on serum TNF-alpha level

In aforesaid studies, we have found that the homeopathic drugs (medorrhinum, silica, sulphur) and indomethacin were found to be potent in ameliorating inflammation in carrageenan and CFA induced arthritis. Thus, these homeopathic drugs were further used for ELISA and Dot-blot analysis. TNF-alpha is a provocative marker to evaluate localized inflammation in the joints. The estimation of serum TNF- α was carried out using ELISA kits. The standard curve of TNF- α was plotted by taking serial dilutions (0, 31.25, 62.5, 125, 250, 500 and 1000) as per kit protocol. Once we inject CFA in left hind paw of animals, we observed significant elevation ($p<0.01$) in circulating TNF-α in the CFA-control animals (Fig. 3) as compared to normal control group. After treating animals with medorrhinum, silica, sulphur significantly ($p<0.001$) depletes serum TNF-α level. Animals treated with indomethacin exhibit significant ($p<0.001$) elevation in serum TNF-α in comparison to the CFA-control animals and that may because of the gastric mucosal destruction due to indomethacin.

Effect of homeopathic drugs on dot blots analysis

Circulating IL-1β and IL-6 level were evaluated by dot blot analysis (Fig. 4). We noted down significant diminution in serum IL-1β and IL-6 expression in medorrhinum, silica, sulphur treated groups in comparison to control but increase in serum IL-1β and IL-6 were examined in indomethacin treated animals in contrast to control, this enhancement was not statistically significant.

Effect of Homeopathic drugs on synovial expression of NF-κB

The immunohistochemistry staining of NF-κB was evaluated in synovial tissue of animals. The CFA-control animals produced momentous elevation in expression of cytokines, and degrading enzymes in synovium. Medorrhinum treated animals showed significant depletion in expression of NF-κB as compared to the CFA-control animals (Fig 5).

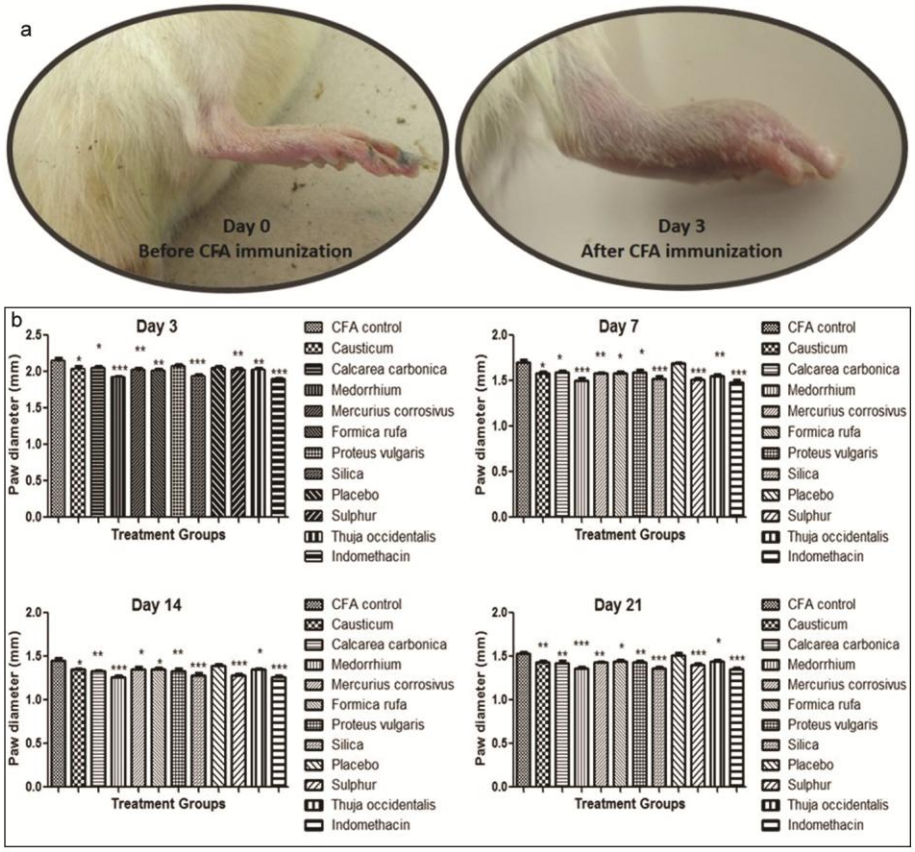
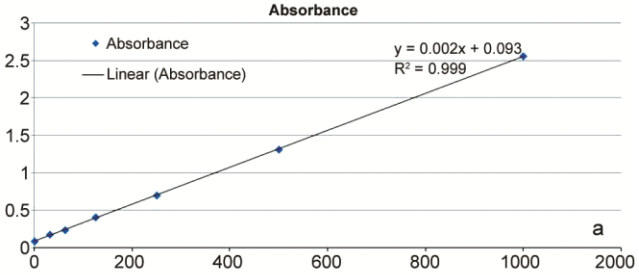


Fig. 2A-B— Effect of Medorrhinum on paw edema in Complete Freund's adjuvant (CFA) induced arthritis in rats before and after CFA immunization; 2B–Effect of administering different homeopathic drugs on Complete Freund's adjuvant (CFA) induced arthritis in rats on Day 3. All values are Mean±SEM (n=6). Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. *p<0.05, **p<0.01 as compared with disease control

Discussion

From the current study we observed that the homeopathic drugs reduce inflammation and attenuate experimental arthritis. Together, it augments the synovial proliferation in CFA-induced model. Further the H&E (Haematoxylin & eosin investigation reinforces the evidence on reduction of inflammation and damage in bones.

As discussed, prior, inflammatory disorders like RA is sustained, chronic disease of joints whose aetiology is still undefined. It is characterized by inflammation of synovial membrane, pain, disparaging transformations in cartilage and bone that restricts the mobility of joints. The synovial fibroblasts and leukocytes in joints secretes numerous pro-inflammatory mediators like tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-8, IFN-γ that contributes inflammation and joint damage[1]. There are lots of existing substantiation which anticipated that pro-inflammatory cytokines such as TNF- α and IL-1 plays a prime role in the



S.No	Treatment groups	Serum TNF- α level (pg/ml)
1.	CFA-control	67.67±1.22
2.	Indomethacin	126.2±1.72***
3.	Medorrhium	24.83±2.50***
4.	Silica	26.50±2.32***
5.	Sulphur	29±1.29***

b

Fig. 3 — Analysis of serum TNF-α with the help of ELISA. (A) Standard curve of varying conc (0, 31.25, 62.5, 125, 250, 500 and 1000) of TNF. (B) Tabulated serum levels of TNF-α of medorrhinum, sulphur, silica and indomethacin treated groups. All values are Mean±SEM (n=6). Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. *p<0.05, **p<0.01 as compared with disease control

pathogenesis of RA. As they lead to the self-proliferation of synoviocytes and enhance the building of enzymes present in the tissues, results in destruction of cartilage[3]. Also, some other studies put forward that the transcription factor, NF-κB, is also a significant regulator in the joint inflammation and degradation by upregulating levels of many pro-inflammatory genes, such as, IL-1β, IL-6, TNF-α, chemokines and MMPs (Matrix-metalloproteinase)[15,16]. Several other studies discovered the increased NF-κB levels in the synovial joint of animal and human models[17]. In short, we can conclude that in the process of bone erosion NF-κB unregulated the expression of TNF- α, which further elicit the production of other cytokines, chemokines and furthermore, it exerts its arthritogenic efficacy via activation of IL-1. Consequently, IL-1, NF-κB and TNF- α are leading moderators for stimulating process of inflammation and bone erosion. The accompanying occurrence of chronic pain and other collateral symptoms is observed as suggestive underlying immune activity in RA. Therefore, the reduction in inflammation and joint dysfunction are the prime action for the drugs which are used to treat inflammatory disorders such as RA. In the existing investigation, we tested homeopathic drugs (causticum, calcarea, medorrhinum, mercurius, formica, proteus, silica, sulphur, thuja) in experimental models of inflammation and RA. Prediction of inflammatory response, carrageenan induced paw edema model was used and for rheumatoid arthritis, CFA induced adjuvant model was used.

The measurement of inflammation was done by carrageenan induced paw edema model as this exhibit with the early exudative inflammation phases. It is a phlogistic tool for screening of newer drug candidates for inflammation. The carrageenan injection at subplantar surface of hind paws of animals showed a sudden augmentation in paw volume and it is in

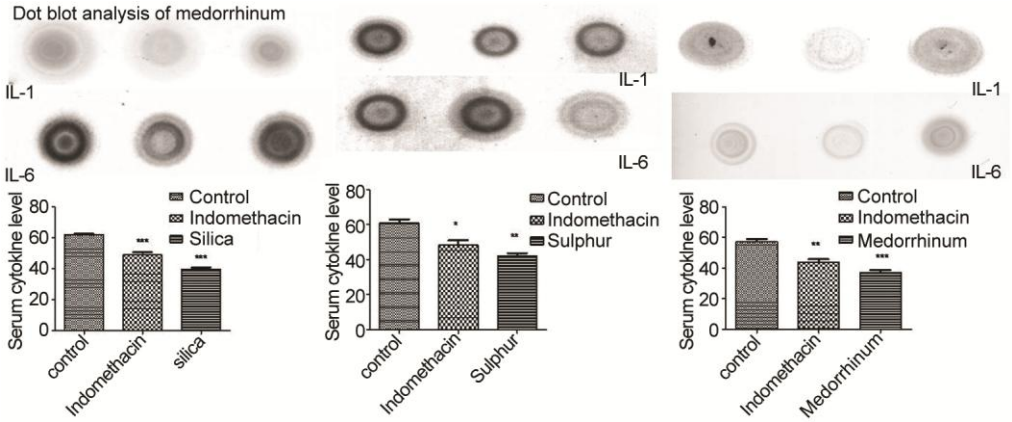


Fig. 4 — Dot blot analysis of IL-1 and IL-6 in medorrhinum, silica and sulphur treated groups. All values are Mean±SEM (n=6). Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. *p<0.05, **p<0.01 as compared with disease control

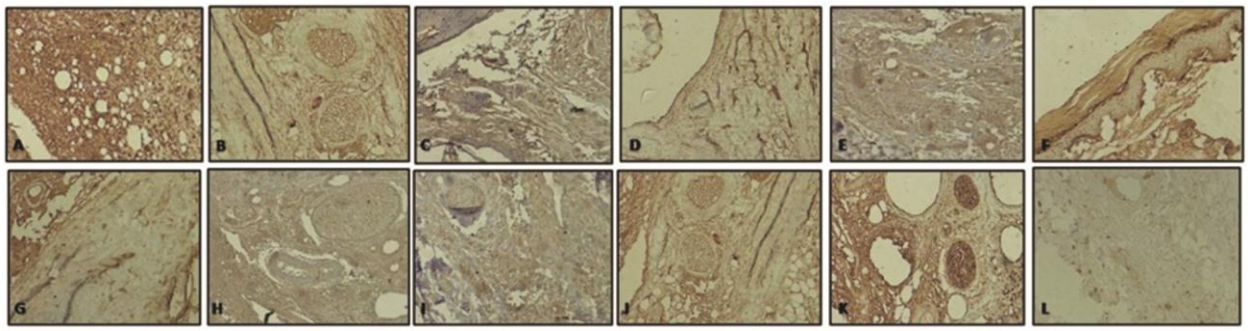


Fig. 5 — Effect of homeopathic drugs on NF-κBexpression in synovial joint of rats in CFA-induced arthritic model. Sections are 6 μm thick and photomicrographs are taken at 10X. Bold arrows = DAB staining (yellow-brown) of the synovial membrane depicting presence of cytokines. (A) CFA control (B) Causticum (C) Calcarea (D) Medorrhinum (E) Mercuris (F) Formica (G) Proteus (H) Silica (I) Placebo (J) Sulphur (K) Thuja(L) Indomethacin. Immunohistochemical analysis of NF-kB in CFA treated groups revealed presence of vacuoles, infiltration of inflammatory cells and some traces of necrosis in ankle joint tissue. However, prominent reversal of joint damage was seen in homeopathic drugs (Medorrhinum, Silica, Sulphur) and indomethacin (Reference drug).

correlation with the vascular permeability and release of histamine, serotonin. 1h later to carrageenan injection the inflammation is turn out to be severe and formation of edema in paws moderately upraise at 4-5 h post carrageenan administration. This is known as the second phase results caused by the release of prostaglandins, kinins and bradykinin in ankle joint tissues accompanied by migration of leukocytes[18]. The inflammatory process exhibited in the present investigation is in close proximity with earlier reports while the dose-dependent hindrance of inflammation by homeopathic drugs from 1-5 h succeeding the initiation of inflammation implied that homeopathic drugs might react on initial and later parts of inflammation. Hence, carrageenan-induced rat paw edema demonstrated momentous anti-inflammatory activity at the tested dose and inhibited joint swelling. The potent activity was seen in medorrhinum, silica, sulphur treated groups among all the homeopathic test groups used in the study. To establish the anti-arthritic activity of the homeopathic drugs, the effectiveness of test drugs in decreasing joint inflammation in CFA induced arthritis was assessed. The experimental model exhibits numerous clinical and immunological features similar to arthritis development in humans. Therefore, for pharmacological screening of anti-arthritic lead candidates this model is widely accepted[14]. The peak inflammatory response elicited by CFA has been reported to correlate with fluid exudation, neutrophil infiltration, release of cytokines and inflammatory mediators such as PGs (Prostaglandins) and histamine[19]. The inflammation produced in ankle joints is linked with formation of granuloma. In this study, there was a consistent decrease in joint swelling. TNF-alpha and other pro-inflammatory cytokines (IL-1, IL-6) display an important task in mediation of inflammation and joint damage in CFA-induced arthritis model. The analysis of aforesaid mediators was carried out by using ELISA and dot blot techniques. It was found that the homeopathic drugs (medorrhinum, silica, sulphur) possess significant ($p<0.05$) anti-inflammatory and anti-arthritic activities. The immunohistochemical analysis of NF-kB also demonstrated the corroborated findings with CFA-induced arthritis model. All the homeopathic drugs have more or less anti-inflammatory activity but the homeopathic drug medorrhinum showed significant effect on pro-inflammatory cytokines (TNF-alpha, IL-1 and IL-6), as evidenced from ELISA and Dot blot analysis. The expression of NF-κB was done by using immunohistochemical analysis and medorrhinum effectively downregulated the increased expression of NF-κB. Histopathological analysis also confirmed the protective effect of medorrhinum, out of all the homeopathic drugs tested to ameliorate the CFA-induced inflammatory changes.

Conclusion

The findings of study revealed that all the homeopathic drugs used in the study have significant effect on inflammation and could be a prospective remedy used for the treatment of inflammatory disorders such as RA. Medorrhinum is found to be an effective anti-arthritic agent which is possibly due to its ability to significantly down regulate the increased expression of pro-inflammatory cytokines and NF-κB. This might be the implying mechanism behind the efficacy of medorrhinum and therefore additional investigations (NF-κB signaling pathway) are invariably required to intricate the effect of medorrhinum on inflammation and bone damage.

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Conflict of Interest

There was no conflict of interest among authors and co-authors.

Authors' Contributions

SS conceived the presented idea, RK performed the experimentation and statistical analysis, SS wrote the manuscript with support from DN, AKK & RKM. All authors discussed the findings and contributed to the final manuscript.

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