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EVALUATION OF ANTI-ARTHRITIC ACTIVITY OF MICROSPONGE FORMULATION IN FREUND'S COMPLETE ADJUVANT-INDUCED ARTHRITIC RAT MODEL

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Keywords:

Microsponge, Control release, SEM, FTIR, DSC, *In-vivo* anti-arthritic activity

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ABSTRACT: The aim of the present study was to improve the release rate of herbal drugs by microsponges prepared through quasi-emulsion solvent diffusion technique using ethyl cellulose and PVA as carriers. The microsponges were characterized by FTIR, DSC and SEM studies followed by the determination of total drug content and entrapment efficiency. Anti-arthritic activity was evaluated by Freund's Complete Adjuvant (FCA) induced arthritic rat model. RA was induced by intradermal injection of 0.1 ml FCA in the left hind paw. The animals were treated with doses 50:50, 70:30, 30:70, and 100:100 mg/kg b.w. of microsponge formulation. Methotrexate at a dose of 10 mg/kg is used as a standard drug. Assessment of paw volume, % inhibition of paw volume, serum parameters for RA factor determination and histopathological examination was also carried out.In this current study, the microsponge formulation (*B. serrata* + *A. barbadensis*) had a better effect on controlling CFA induced arthritis. Formulation codes M1 and M4 showed a good reduction in paw edema and joint thickness. It produced a significant reduction of R_f values comparable to the methotrexate treated group. Therefore prepared microsponge has got definite effect in reducing the inflammatory components as well as the above-cited results also focus on its immunomodulatory role. Hence, the polymeric microsponge-based formulation of herbal drugs would be an anticipation and promising substitute to conventional therapy of rheumatoid arthritis and supports the common belief prevailing in traditional medicines worldwide.

INTRODUCTION: Microsponges are at the forefront of the rapidly developing field of novel drug delivery technology. Microsponge, drug delivery technology, holds a great promise for reaching the goal of controlled and site-specific drug delivery and hence, has attracted wide attention of researchers in managing arthritic conditions.

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Microsponges are polymeric delivery systems composed of porous microspheres. They are tiny sponge-like spherical particles with a large porous surface [1]. Microsponge technology has many favorable characteristics, which make it a versatile drug delivery vehicle [2].

Microsponge Systems are based on microscopic, polymer-based microspheres that can suspend or entrap a wide variety of substances and can then be incorporated into a formulated product such as a gel, cream, liquid, or powder. The outer surface is typically porous, allowing a sustained flow of substances out of the sphere [3].

Microsponges are porous, polymeric microspheres that are used mostly for topical use and have recently been used for oral administration. Microsponges are designed to deliver a pharmaceutically active ingredient efficiently at the minimum dose and also to enhance stability, reduce side effects, and modify drug release [4].

Arthritis is a painful swelling of joints, and it is a common disease affecting a large population [5]. Osteoarthritis and rheumatoid arthritis are most common. Osteoarthritis is a degenerative joint disease occurring chiefly in older people and rheumatoid arthritis is an autoimmune disorder of unknown etiology [6]. In complementary and alternative medicines such as Ayurveda (herbs) and acupuncture are most commonly used for the treatment of many systemic disorders [7]. Chopra *et al.*, reported around 68% of patients with chronic rheumatic disorders have sought relief using an alternative system of medicine and demonstrated the clinical efficacy of herbal formulation for the treatment of osteoarthritis of the knees [8, 9].

Many herbs and herbal medicines have been used since time immemorial to cure many disorders/ diseases, including arthritis. Plants are the richest sources of pharmaceutical lead molecules, and their contribution to the drug discovery process is remarkable. The objective of the present study is to formulate a microsponge formulation and evaluate its anti-arthritic potential in animals. The microsponge was formulated using the herbs which have known anti-arthritic effects at a particular ratio to enhance the pharmacological activity of individual herb and reduce the dose of single plant extract. In traditional system of Indian medicine combined extract of individual plants rather than individual ones to achieve maximum therapeutic efficacy [10].

Powder extract of resins of *Boswellia serrata* and leaves of *Aloe barbadensis* were used in the microsponge formulation. The preliminary acute toxicity of microsponge and individual plant extract showed no significant toxic effects upto 2000 mg/kg in rodents [11]. In light of the results of the literature studies on individual extracts, it was found to have good anti-inflammatory and anti-arthritic activities [12, 13]. With this background, the present study was planned to screen anti-arthritic effect of a microsponge constituting powder extract of resins of *B. serrata* and leaves of *A. barbadensis* against Freund's complete adjuvant (FCA) induced arthritis in Male Wistar rats.

MATERIALS AND METHODS:

Experimental Animals: The experiment protocol described in the present study was approved by the Institutional Animal Ethics Committee (IAEC) (Approval No: SGRS/IAEC/08/2019-20) and with permission from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No: 311/PO/ReBi/S/ 2000/CPCSEA). Forty-two (42) Male albino rats (200–250g) of wistar strain were procured from the Crystal Biological Solution, Preclinical Animal Testing research center, Handewadi. These rats were kept under standard animal house conditions under relative humidity and temperature. They are provided with free access to water and a standard pellet diet. Prior 7 days to the start of the experiment, the rats were adjusted to the animal house environment. Experimental procedures used for the study were approved, and the guidelines are followed strictly throughout the experimental period.

Plant Material: Herbal extract powders of *Boswellia serrata* (Shallaki) and *Aloe barbadensis (Aloe vera)* are obtained as a gift sample from Sane Guruji Hospital and Ayurvedic Medical Store, Hadapsar, Pune. The collected plant extract was identified and authenticated at the Department of Pharmacognosy, S.G.R.S. College of Pharmacy, Saswad, Pune.

Chemicals: Complete Freund's adjuvant was procured from Sigma-Aldrich. Standard drug Methotrexate (Imutrex) was procured from Cipla Pharmaceuticals. Eudragit RS 100, Dichloromethane, Polyvinyl Alcohol (PVA) were purchased from the research lab center, Pune. The laboratory chemicals other than mentioned above used in the study were of analytical reagents grade.

Formulation of Microsponge Formulation: Microsponges are prepared by a Quasi-emulsion solvent diffusion method [14]. Two-Step Process –1) An internal phase containing polymer such as Eudragit RS 100 which is dissolved in dichloromethane. Then, the drug is slowly added to the polymer solution & dissolved under ultra-

sonification at 35 °C & a plasticizer such as Glycerol is added in order to aid the plasticity. 2) The inner phase is then poured into an external phase containing polyvinyl alcohol & distilled water with continuous stirring for 2 h. Then the mixture is filtered to separate the microsponges. The product (microsponges) was washed & dried in an air heated oven at 40 °C for 24 h (**Table 1** and **Fig. 1** and **2**).

TABLE 1: TABLE REVEALING THE MASTER FORMULA FOR MICROSPONGE FORMULATION

S. no.	Ingredients	M1	M2	M3	M4
1	Drug BS + AB (mg)	50:50	70:30	30:70	100:100
2	Eudragit RS 100 (mg)	100	100	100	100
3	Dichloromethane (ml)	20	20	20	20
4	Glycerol	1	1	1	1
5	PVA (mg)	500	500	500	500
6	Distilled Water (ml)	100	100	100	100

BS = *Boswellia serrata*, AB = *Aloe barbadensis*, PVA = Polyvinyl Alcohol

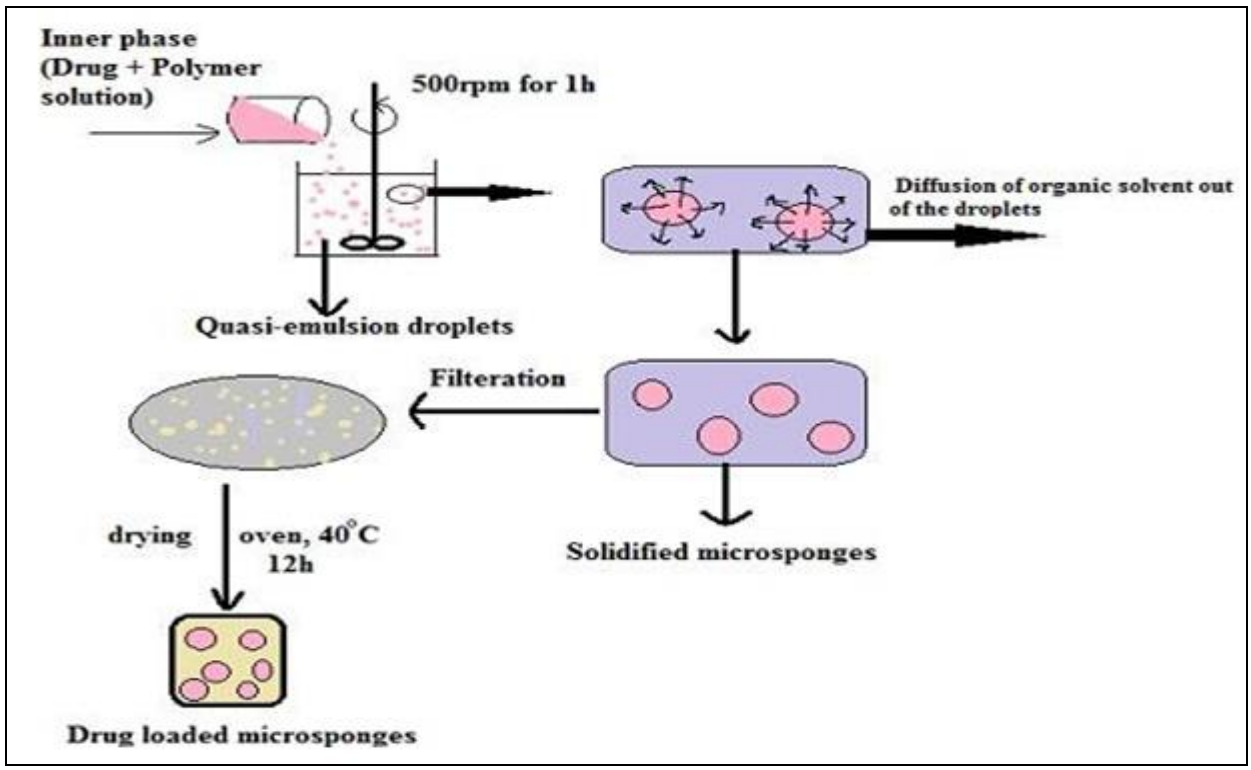

FIG. 1: QUASI-EMULSION SOLVENT DIFFUSION METHOD

FIG. 2: FORMATION OF SPONGE-LIKE MICROSPHERES

Once the formulation was prepared, characterization was done by determining particle size, percent yield, drug content, Fourier Transform Infrared (FTIR), surface morphology by Scanning Electron Microscopy (SEM), and *in-vivo* study.

***In-vivo* Anti-arthritic Activity of Microsponge Formulation:**

Experimental Design: Fourty-two (42) rats were put into seven (7) groups of six (6) rats each as follows:

Group I: Was not induced and served as Normal Control (NC) receiving vehicle 5% CMC.

Group II: Was induced with rheumatoid arthritis using FCA 0.1 ml in subplanter region and given distilled water. This was the Arthritic Control (AC).

Group III: FCA 0.1 ml in subplanter region on day 1 + [Methotrexate (10 mg/kg, p.o) from day 1 to 12]. This was the Standard Control (SC).

Group IV: FCA 0.1 ml in subplanter region on day 1 and treated with Microsponge formulation [BS (50mg/kg) & AB (50mg/kg, p.o) from day 1 to day 12] .

Group V: FCA 0.1 ml in subplanter region on day 1 and treated with Microsponge formulation [BS (70mg/kg) & AB (30mg/kg, p.o) from day 1 to day 12].

Group VI: FCA 0.1 ml in subplanter region on day 1 and treated with Microsponge formulation [BS (30mg/kg) & AB (70mg/kg, p.o) from day 1 to day 12] .

Group VII: FCA 0.1 ml in sub-planter region on day 1 and treated with Microsponge formulation [BS (100mg/kg) & AB (100mg/kg, p.o) from day 1 to day 12].

Induction of Arthritis: The animals were injected with 0.1 ml of Freund's complete adjuvant into the sub-plantar surface of the left hind paw. Drugs were administered orally, once a day commenced on the day of injection of adjuvant and continued for 12 days. The assessment of the change in the inflammatory reaction was made by measuring the paw volume plethysmographically on 0, 5th & 21st day after injection of Complete Freund's adjuvant (CFA) [15]. The percent inhibition of paw volume of treated rats was evaluated by using following formula [16]:

$$\% \text{ Inhibition} = (Vt - Vo)_{control} - (Vt - Vo)_{test} \times 100 / (Vt - Vo)_{control}$$

Where, Vt = paw volume at time t, Vo = paw volume at time 0

Biochemical Analysis: After the end of the experimental period (*i.e.* on 21th day), the rats were sacrificed and the 8ml blood was withdrawn through retro-orbital route from animals and serum was separated. The serum was used for estimation of parameters such as RA (Rheumatoid Arthritis Factor) [17].

Histological Analysis: The knee joints were removed from arthritic rats are engrossed in formaldehyde (10%) for 24 h followed by immersion in 5% formic acid. Then the tissues are processed, sectioned and embedded at 5m thickness. The sections were stained in Haematoxylin and Eosin stain and viewed under a light microscope, which gives the localization of inflammatory cells present and destruction of joints [18].

Statistical Analysis: Results were presented as Mean ± SEM of six animals. The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test. The significance difference, if any, among the groups at $p < 0.05$ was considered statistically significant, $p < 0.01$ was considered statistically highly significant.

RESULTS AND DISCUSSION: Quasi-emulsion solvent diffusion method has been used for the development of BS + AB microsponges. The microsponges were characterized by various parameters.

Characterization and Evaluation of Microsponges Formulation:

Particle Size Analysis of Microsponges: The particle size of the microsponge was determined by optical microscopy [19,] and the microsponges were found to be uniform in size. The average particle size of all formulations ranges from 23.5 µm to 42.7 µm, which is in increasing order due to the increase in the concentration of the drug.

In the M4 formulation, it was observed that as the ratio of drug to polymer was increased (*i.e.,* 2:1), the particle size decreased. This could probably be due to the fact that in a high drug to polymer ratio, the amount of polymer available per microsponge was comparatively less. Probably in high drug-polymer ratios, less polymer amounts surround the drug, and reducing the thickness of polymer wall, and microsponges with smaller size were obtained [20]. By performing the particle size analysis, it is concluded that the formulation has the particle size varies with the concentration of drug-polymer ratio (as shown in **Table 2**).

TABLE 2: TABLE REVEALING THE RESULTS OF PARTICLE SIZE ANALYSIS OF MICROSPONGES

S. no.	Formulation Code	Drug Polymer Ratio	Particle Size (µm) (mean ± S.D) n=3	Total Drug Content (%) (mean ± S.D) n=3	Entrapment Efficiency (%) (mean ± S.D) n=3
1	M1	1:1	42.7 ± 1.80	45.94 ± 1.5	70.3 ± 1.08
2	M2	1:1	37.4 ± 0.81	57.38 ± 2.26	87.8 ± 1.57
3	M3	1:1	35.1 ± 1.65	61.40 ± 3.01	93.95 ± 2.73
4	M4	2:1	23.5 ± 1.40	86.83 ± 1.36	88.56 ± 2.09

Percentage Yield (%): It is calculated to know about the efficiency of any method; thus it helps in selection of an appropriate method of production [21].

After the preparation of formulations, the Practical yield was calculated as Microsponges recovered from each preparation in relation to the sum of starting material (Theoretical yield). It can be calculated using the following formula [22]:

Percentage yield = Practical yield × 100 / Theoretical yield (drug + polymer)

The percentage yield of microsponges is shown in **Table 3**. It is found that on increasing the drug to polymer ratio, the increase in production yield. The loss of product was due to the formation of some agglomerates and polymer adherence to the container as a result of vicious nature of slurry.

TABLE 3: TABLE REVEALING THE RESULTS OF PERCENT YIELD

S. no.	Formulation Code	Theorotical Yield (mg)	Practical Yield (mg)	Percentage Yield (%) (mean ± S.D) n=3	Stirring (RPM)
1	M1	200	119	59.5± 0.75	500
2	M2	200	148	74.0± 1.45	500
3	M3	200	163	81.5± 2.81	500
4	M4	300	277	92.3± 1.72	1000

Total Drug Content and Entrapment Efficiency: The weighed amounts of drug-loaded microsponges (10 mg) were dissolved in 10 mL methanolic phosphate buffer solution (pH 7.4) with occasional stirring. 1 mL of the above sample was appropriately diluted with methanolic phosphate buffer, and the absorbance was taken at 660 nm against blank using methanolic phosphate buffer solution where the value of E1% is 0.306. The total drug content was calculated as follows [23]:

Total drug content = Abs × dilution factor × 100 / E 1% ...(1)

The drug entrapment efficiency (%) was calculated as (Eq. 2):

%EE = TDC / amount of drug added × 100 ... (2)

Where, TDC is the total drug content in microsponges and % EE is the percentage of entrapment efficiency of the microsponges.

The total drug content and entrapment of the drug depend on the successful molecular association of the drug with the polymers [24]. TDC and EE of the microsponges were found in the range of 45.94 ± 1.5 to 86.83 ± 1.36 % and 70.3 ± 1.08 to 93.95 ± 2.73% of different batches **Table 2**. The values of TDC were found maximum for the formulation M4 having the drug to polymer ratio of 2:1. A drop in EE was observed on further increasing drug/ polymer ratio for formulation M4. The probable reason for this decrease in EE could be that the optimum concentration of polymer is not available to coat or entrap the drug molecules.

Morphology Determination by Scanning Electron Microscopy (SEM): Scanning electron microscopy (SEM) was used to determine the Morphology of the prepared microsponges. SEM is useful for characterizing the morphology and size of microscopic specimens with a particle size as

low as 10^{-10} to 10^{-12} grams. The sample was placed in an evacuated chamber and scanned in a controlled pattern by an electron beam. Interaction of the electron beam with the specimen produces a variety of physical phenomena that, when detected, are used to form images and provide elemental information about the specimens [25].

It was observed that the microsponges were spherical and uniform with no drug crystals on the surface. The shape of the microsponges affects the surface area and surface area per unit weight of spherical microsponges [26]. The irregular shape of the particles may affect the dissolution rate present in the dissolution environment.

Physical appearance showed white to almost white microsponge particles were obtained by quasi emulsion solvent diffusion method. The images obtained from SEM revealed that the particles were spherical in shape with a porous structure which indicates that microsponge formulations were prepared correctly (as shown in **Fig. 3** and **5**). Fractured images **Fig. 4** showed that due to evaporation of solvent from the surface of micro-sponges, pores were induced.

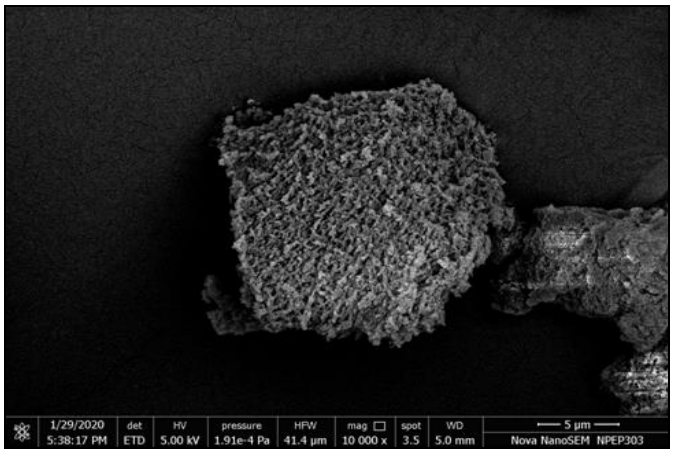

FIG. 3: IMAGE SHOWING SINGLE SPHERICAL MICROSPONGE AT 10000X MAGNIFICATION

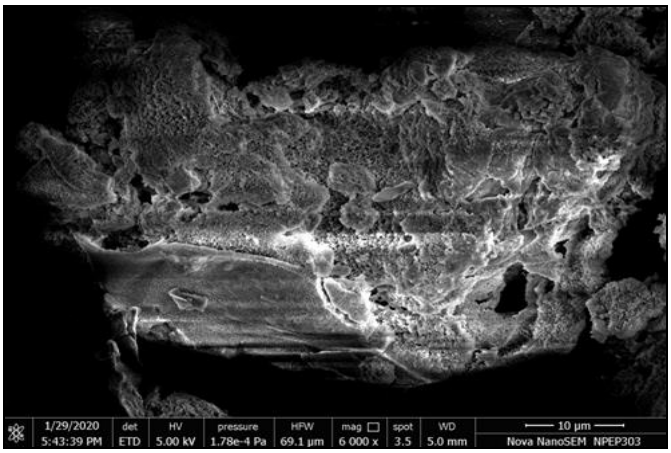

FIG. 4: SEM IMAGE OF FRACTURED MICROSPONGE AT 6000X MAGNIFICATION

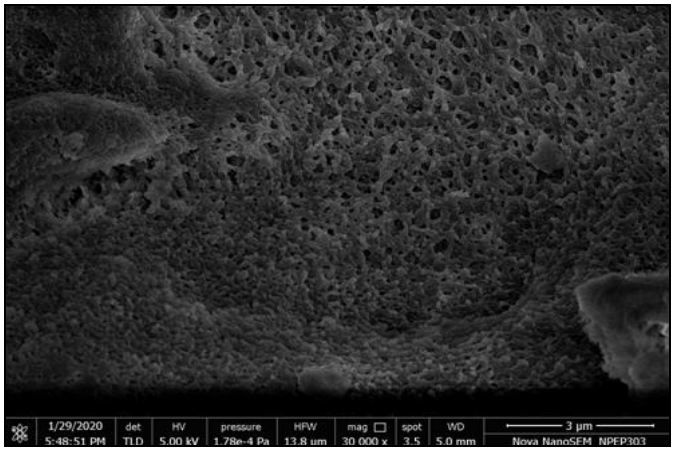

FIG. 5: IMAGE SHOWING POROUS SURFACE MORPHOLOGY OF MICROSPONGE AT 30000X MAGNIFICATION

Fourier Transform Infrared (FT-IR) Studies: IR spectra of the pure drug, other excipients and formulations were obtained and compared. In the present study, the potassium bromide (KBr) pellet method was employed. The samples were thoroughly mixed with dry powder of potassium bromide and scanned from 4000-400 cm^{-1} by FT-IR spectrophotometer (Model number 8400S Shimadzu, India) [27].

The IR spectra of pure herbal drug and formulation are shown in **Fig. 6**. The peak at 3124cm^{-1} indicates O-H stretching, 2924cm^{-1} for the C-H stretching, 1975cm^{-1} for the aromatic C=C stretching, 1681cm^{-1} for the N-H bending, 1453cm^{-1} for the C-H bending. These are the major spectral peaks of the drug. All these peaks were present in the formulation, which confirms that the drug did not interact with the excipients.

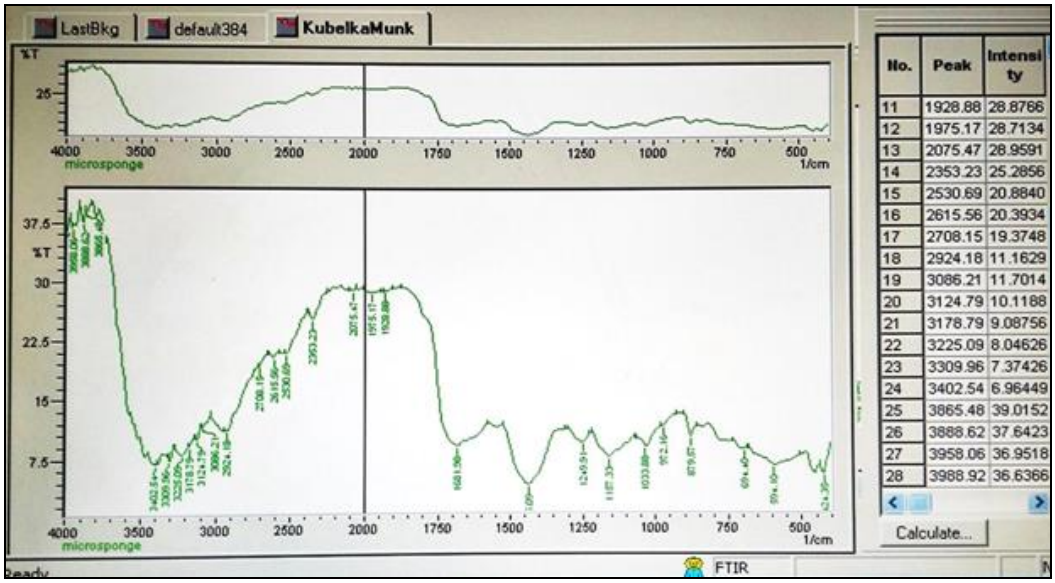

FIG. 6: FTIR SPECTRA OF PURE DRUG AND MICROSPONGE FORMULATION

Differential Scanning Calorimetry (DSC): Differential scanning calorimetry was performed by differential scanning calorimeter (Model number eSTAR Mettler Toledo) to obtain suitable thermo grams. The accurately weighed sample was placed in an aluminium pan and an empty aluminium pan was used as a reference. The experiment was performed under nitrogen flow, at a scanning rate 10°C/min in the range of 50-350 °C **Fig. 7**.

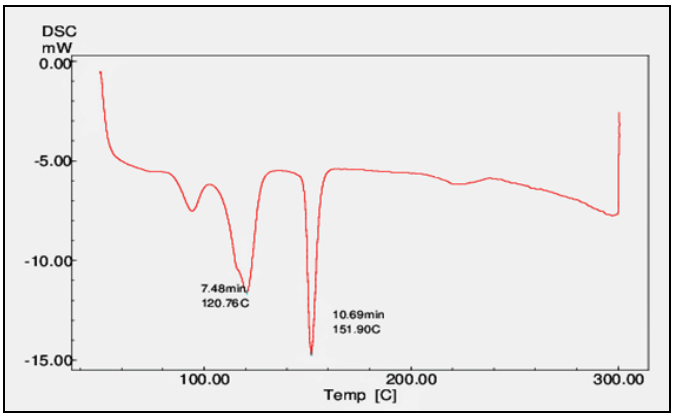

FIG. 7: DSC THERMOGRAM OF MICROSPONGE FORMULATION

From the thermograms of DSC study obtained, it was observed that there was no interaction between the pure herbal drugs and polymer as well as crystalline nature of both drugs BS + AB remains thermally stable upto the final formulation. DSC is used to detect possible interactions between a drug entity and the excipients in its formulation to ensure the success of the subsequent stability studies [28]. *B. serrata* shows a sharp endothermic peak at around 151 °C while *A. barbadensis* shows a peak at 120 °C (as shown in **Fig. 7**) corresponding to its melting point as prescribes in references. This peak sill present in its microsponage formulation, which certified that the drug maintains its crystal form and it's stability.

***In-vivo* Anti-Arthritic Activity:**

Acute Oral Toxicity Study: A detailed study on the acute oral toxicity of these plants was already reported by us revealed that the BS and AB extracts were non-toxic up to the dose of 2000 mg/kg [11, 12].

Paw Volume: After administration of the herbal microsponge formulations (M1, M2, M3 & M4) from 1 to 12 days, changes in rat paw volume were recorded on 0^{th}, $5^{th,}$ and 21^{th} days **Table 4** and **Fig. 8**. The arthritic control groups showed signs of arthritis development as seen by the increase in paw volume. Significant ($p<0.001$) reduction in rat paw volume was observed in standard methotrexate group, and microsponge treated groups on 21^{st} day after FCA induction. When compared with the standard methotrexate group, microsponge formulation with M1 (50:50 mg/kg) and M4 (100:100 mg/kg) showed the most significant decreased in paw volume results. While M2 (70:30 mg/kg) and M3 (30:70 mg/kg) formulations were found to be less significant than the standard drug methotrexate.

FCA-induced arthritis is the most widely used model in which the clinical and pathological changes are comparable with those seen in human rheumatoid arthritis [29].

TABLE 4: EFFECT OF MICROSPONGE FORMULATION ON PAW VOLUME IN FCA INDUCED ARTHRITIC RATS

Group	Treatment (mg/Kg)	Paw volume (ml)		
		Day 0	5th Day	21th Day
I	Normal control	0.84 ± 0.006	0.87 ± 0.008	0.86 ± 0.006
II	Arthritic control	0.96 ± 0.01	1.92 ± 0.004***	2.78 ± 0.007***
III	Methotrexate (10, p.o.)	0.85 ± 0.008	1.38 ± 0.018***	1.27 ± 0.01***
IV	M1 Formulation (50:50)	0.83 ± 0.015	1.24 ± 0.006***	1.03 ± 0.01***
V	M2 Formulation (70:30)	0.84 ± 0.014	1.47 ± 0.013***	1.16 ± 0.008***
VI	M3 Formulation (30:70)	0.91 ± 0.008	1.58 ± 0.01***	1.36 ± 0.02***
VII	M4 Formulation (100:100)	0.81 ± 0.018	1.10 ± 0.007***	0.95 ± 0.017***

Values are expressed as mean ± SEM (n =6). *** P <0.001 compared with arthritic control. Data was analyzed using one-way ANOVA followed by Dunnett's multiple comparison test

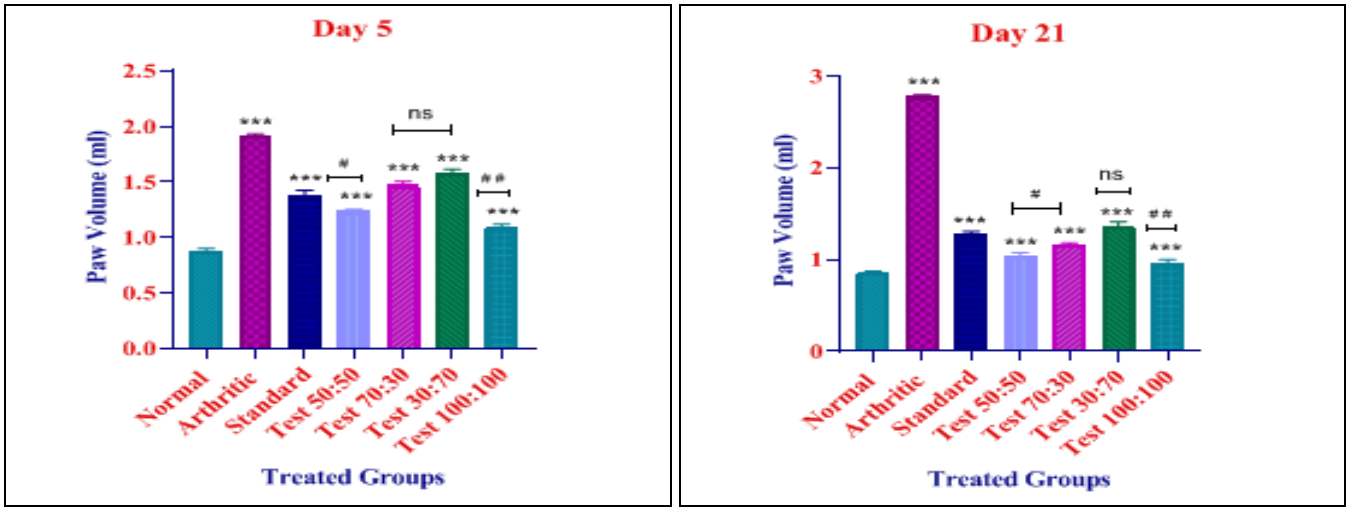

FIG. 8: EFFECT OF MICROSPONGE ON PAW VOLUME IN FCA INDUCED ARTHRITIC RATS. Each value represents the mean± SEM (n =6). Data was analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. *** P <0.001 compared to arthritic group. # P <0.05; ##P <0.01 compared to standard group. ns: Not significant

Percent Inhibition of Paw Edema: The activity of BS+AB Microsponge with formulation code M1, M2, M3 & M4 and Methotrexate to inhibit arthritic paw edema was examined against the CFA-control group and found to be significant at p<0.001 respectively **Table 5** and **Fig. 9**. The highest percent of inhibition was expressed by M4 at a dose of 100: 100 mg/kg, especially at chronic phase at 90.37% and also more active than Methotrexate (78.12%).

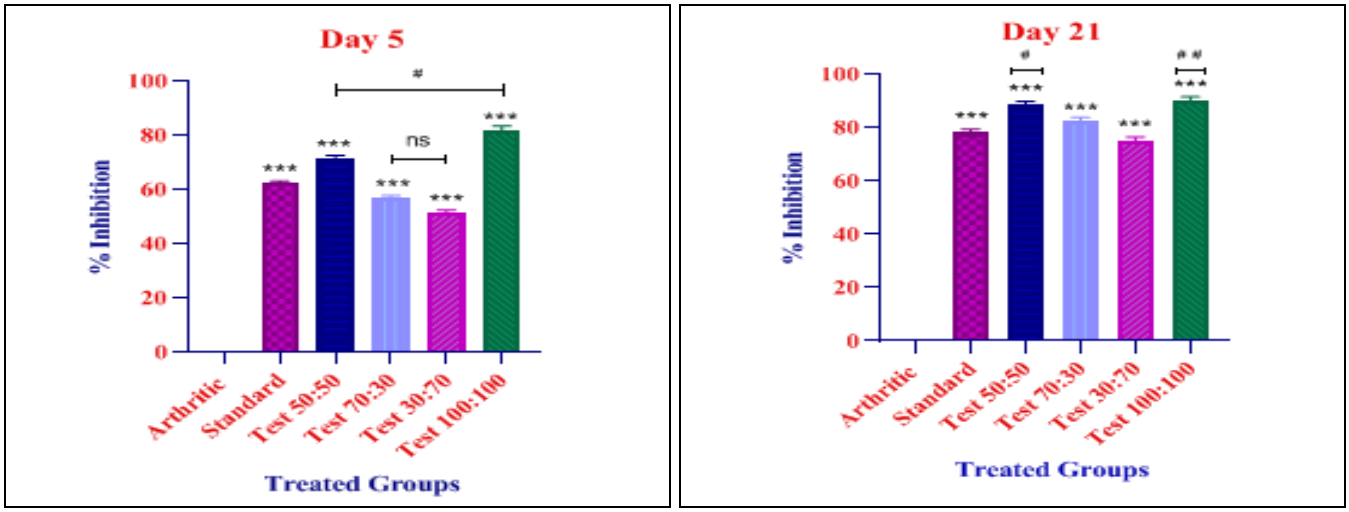

FIG. 9: INHIBITION OF RAT PAW EDEMA BY REFERENCE STANDARD (METHOTREXATE) AND TEST (MICROSPONGE) FORMULATIONS. Each value represents the mean ± SEM (n =6). Data was analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. ***P <0.001compared to arthritic group. #P <0.05, ##P <0.01 compared to standard group. ns: Not significant

TABLE 5: EFFECT OF MICROSPONGE FORMULATION ON PERCENTAGE INHIBITION OF PAW EDEMA IN FCA INDUCED ARTHRITIC RATS

Group	Treatment (mg/Kg)	Percentage inhibition of paw edema in rats (%)	
		5th Day	21th Day
II	Arthritic control	0	0
III	Methotrexate (10, p.o.)	62.50 ± 0.63***	78.12 ± 1.30***
IV	M1 Formulation (50:50)	71.89 ± 0.85***	88.58 ± 1.29***
V	M2 Formulation (70:30)	57.06 ± 0.71***	82.66 ± 1.10***
VI	M3 Formulation (30:70)	51.73 ± 0.83***	75.17 ± 1.39***
VII	M4 Formulation (100:100)	81.99 ± 1.49***	90.37 ± 1.14***

Values are expressed as mean ± SEM (n =6). ***P <0.001 compared with arthritic control. Data was analyzed using one-way ANOVA followed by Dunnett's multiple comparison test.

Estimation of Serum RA Level: At the end of 21 day of treatment schedule, the RA level in group-III, IV, V, VI and group VII was found to be 8.41 ± 0.16, 5.9 ± 0.04, 11.2 ± 0.09, 12.13 ± 0.17 and 2.08 ± 0.11 IU/L respectively. This indicates, significant (***p<0.001) decreased in RA level, compared with group II *i.e.* 18.71 ± 0.16 (arthritic control). More significant decrease in RA level was observed in group IV and group VII, when compared with group- III **Table 6** and **Fig. 10**.

TABLE 6: EFFECT OF MICROSPONGE FORMULATION ON RHEUMATOID FACTOR (RA)

Group	Treatment (mg/kg)	RA level (IU/L)
I	Normal control	1.04 ± 0.01
II	Arthritic control	18.71 ± 0.16***
III	Methotrexate (10, p.o.)	8.41 ± 0.16***
IV	M1 Formulation (50:50)	5.9 ± 0.04***
V	M2 Formulation (70:30)	11.2 ± 0.09***
VI	M3 Formulation (30:70)	12.13 ± 0.17***
VII	M4 Formulation (100:100)	2.08 ± 0.11***

Values are expressed as mean ± SEM (n =6). ***P <0.001 compared with arthritic control. Data was analyzed using one-way ANOVA followed by Dunnett's multiple comparison test

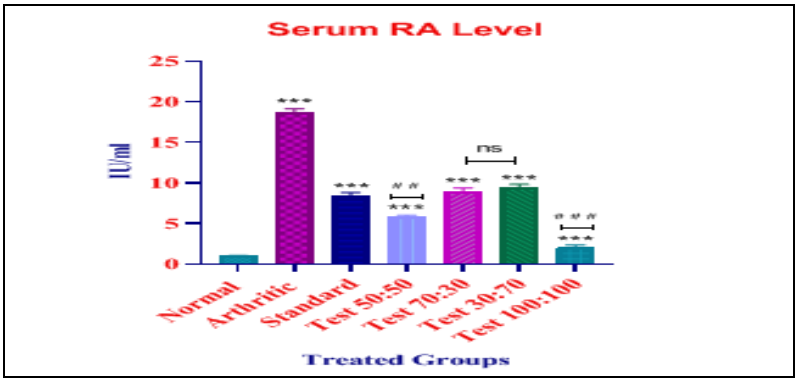

FIG. 10: EFFECT ON SERUM RA LEVEL BY REFERENCE STANDARD (METHOTREXATE) AND TEST (MICROSPONGE) FORMULATIONS. Each value represents the mean± SEM (n =6). Data was analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. ***P <0.001 compared to arthritic group. ##P <0.01; ###P <0.001 compared to standard group. ns: Not significant

Histopathology of Knee Joints: The histological assessment of tissue sections from rat knee joint was performed on day 21th of treatment. The joint histological architecture of rats nontreated with CFA served as a normal (Group I). The histopathology of paw tissue **Fig. 11** revealed that adjuvant-induced arthritic rats (Group II) showed severe edema formation, cell infiltration, vascular dilatation, and disorganization of fibroblasts and collagen fibers. The methotrexate-treated group (Group III) shows moderate edema formation and cellular infiltration and the Microsponge-treated groups (Group IV, V, VI & VII) showed a relatively good organization of the joint structure without edema formation and vasodilatation. Microscopic examination of the knee joint (bone showed minimal multifocal infiltration of inflammatory cells in animal (Group 2) belonging to disease control when compared with the normal control group. However, other animals did not show any abnormality of pathological changes when compared with the normal control group.

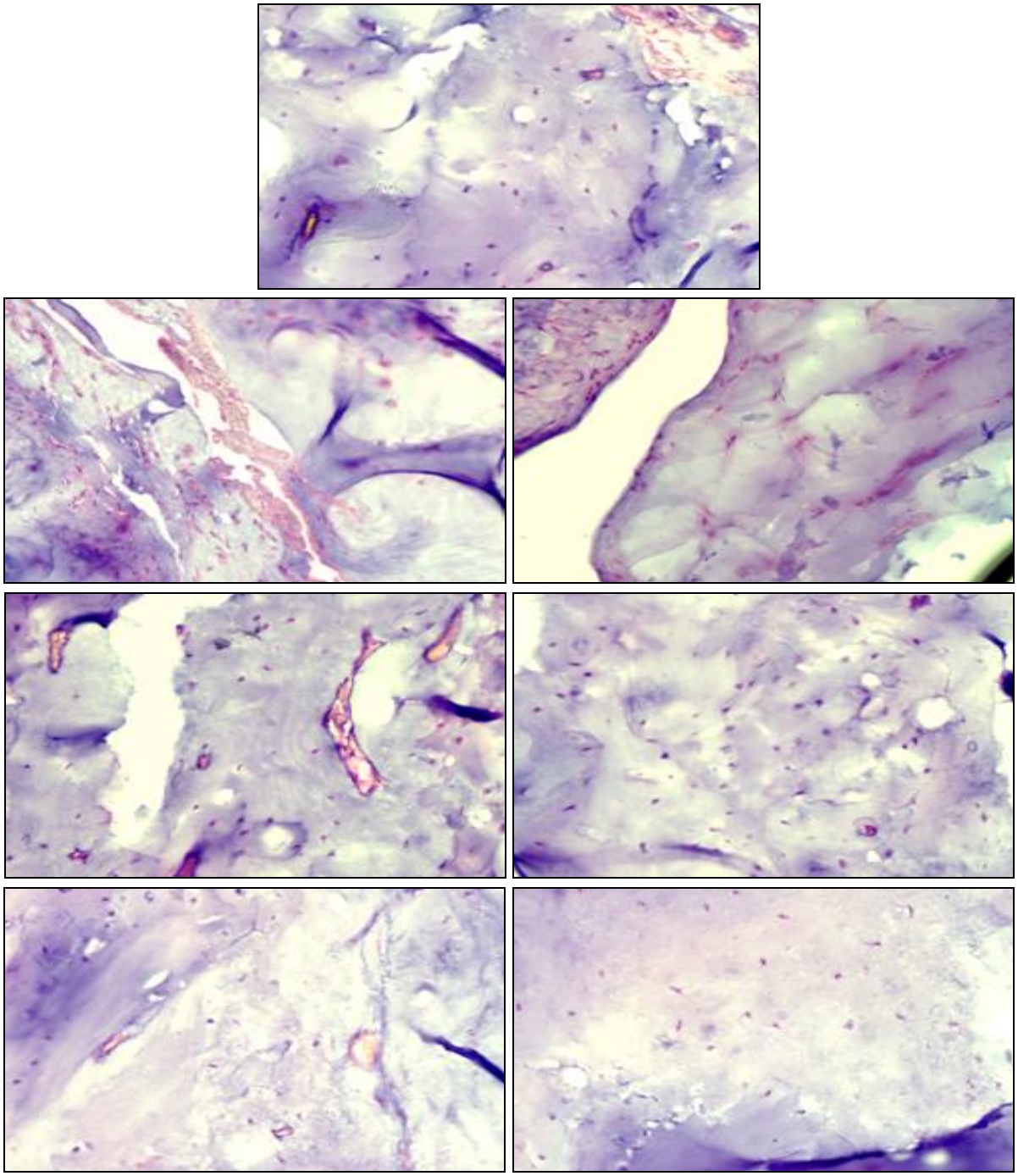

FIG. 11: HISTOPATHOLOGY OF KNEE JOINTS

CONCLUSION: The BS+ AB microsponges were prepared by simple, reproducible and rapid quasi-emulsion solvent diffusion method and were evaluated for its different parameters, which revealed many interesting results for efficient preparation of the microsponges. Based on the above results, it could be concluded that microsponge formulation, a combination of two herbal plants, exert a significant anti-arthritic effect due to different types of active principles each with a single or a diverse range of biological activities, which serves as a good adjuvant in the present foundation of these pathological conditions. Further clinical studies are needed to establish its safety and usefulness medically.

It is concluded that microsponges drug delivery system has become highly competitive and rapidly evolving technology, and more and more research are carrying out to optimize the cost-effectiveness and efficacy of the therapy. Microsponge delivery systems can precisely control the release rates or target drugs to a specific body site have a vast impact on the health care system. A microsponge

delivery system can release its active ingredient on a timer mode and also in response to other stimuli. Therefore, microsponge has got a lot of perspective and is a very rising field which is needed to be examined, and it is probable to become a precious drug delivery matrix substance used for different therapeutic applications in the upcoming future.

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