Research article Open-Access

BCHD
Bioactive Compounds in Health and Disease

Pain and Inflammation Management: Part-II Clinical Investigation of a Topical Ayurvedic Cream called HerboCare or HerboJoint™.

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Submission Date: June 23rd, 2023; **Acceptance Date**: December 6th, 2023; **Publication Date**: December 11th, 2023

Please cite this article as: Bordoloi B. K., Saini K. S., Sarma P. B., Jolly K., Bordoloi R. K., Kandimalla R., Gajbhiye R. L., Sengupta S. Pain and Inflammation Management: Part-II Clinical Investigation of a Topical Ayurvedic Cream called HerboCare or HerboJoint™. *Bioactive Compounds in Health and Disease* 2023; 6(12): 338-350. DOI: https://www.doi.org/10.31989/bchd.v6i12.1139

ABSTRACT

Background: Joint pain and chronic inflammation pose significant health challenges, particularly among the elderly population. Current treatments often offer only temporary relief and are associated with potential side effects. Ayurvedic botanical herbs and oils, known for their phytochemical-rich, anti-inflammatory properties, hold promise in addressing these issues.

Objective: This study aimed to investigate the clinical efficacy of HerboCare or HerboJoint™, a topical Ayurvedic cream, in alleviating muscle discomfort, inflammation, and joint pain, along with its impact on inflammatory cytokines, particularly TNF-α on human subjects. While HerboCare or HerboJoint had a very low content of Menthol, HerboJoint-Plus (not reported in this study) was formulated with a much higher content of USP grade Menthol.

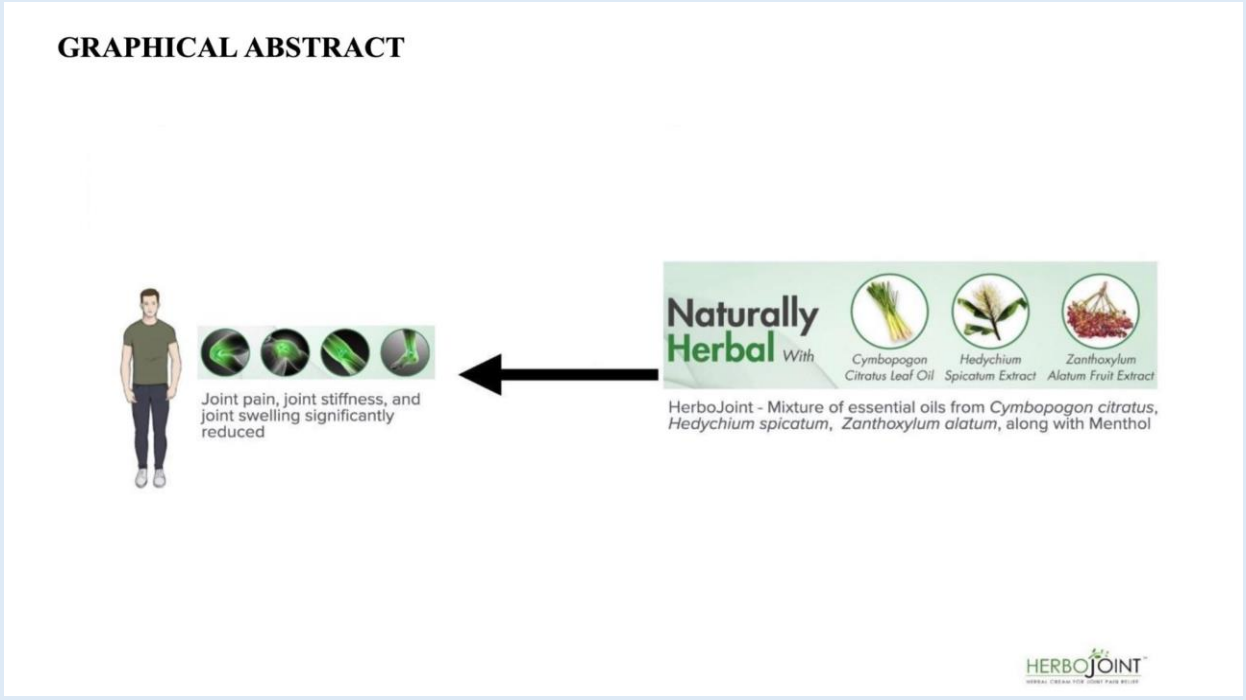
Methods: HerboCare or HerboJoint™ cream, formulated with essential oils from *Cymbopogon citratus*, *Hedychium spicatum*, *Zanthoxylum alatum*, and Menthol (Mentha arvensis), was evaluated in two sets of clinical trials over a 90-day period. Patients with joint pain were divided into different treatment groups, and their pain, swelling, stiffness, and inflammatory marker TNF-α were assessed.

Results: In the first set of trials, HerboCare or HerboJoint™ significantly reduced joint pain (29%), joint swelling (31%), and joint stiffness (60%) in patients. In the second set, TNF-α levels were reduced by 38% in the HerboCare or HerboJoint™-only group, 63% in the HerboCare or HerboJoint™ and prescribed formulation group, and 71% in the group receiving only the prescribed formulation. These results underscored the cream's efficacy in mitigating inflammation.

Conclusion: HerboCare or HerboJoint™, formulated with Generally Recognized as Safe (GRAS)-affirmed botanical ingredients, demonstrates clinical effectiveness in relieving joint pain and inflammation. This Ayurvedic approach offers a safe and cost-effective alternative for managing these debilitating conditions.

Key words: Muscular discomfort, Joint Pain, Essential Oils; Menthol; Inflammatory Cytokines; Clinical Study

GRAPHICAL ABSTRACT



INTRODUCTION:

Joint pain and chronic inflammation are the most frequent functional bone joint disorder in middle aged to older men and women, and especially in elderly population [1-2]. This is characterized by a plethora of changes including gradual changes in the function and structure of periarticular bone, bone hypertrophy, crepitus, deformation of joint shape, degradation of joints, limitation of movement, loss of articular cartilage, formation of osteophytes, and synovial proliferation.

These cascades of mechanistic events lead to a sequence of degradation, resulting in compromised changes in mobility, flexibility, gait, and balance problems [1-2]. Thus, if left untreated, this condition frequently lead to unbearable joint pain and chronic inflammatory responses. Ultimately, it leads to physical disability barring heavy lifting and knee bending activities. This also induces subchondral bone sclerosis in elderly population, a debilitating condition [3]. The CDC reports that "Arthritis will grow as the population grows and changes". During 2013-2015, approximately 22.7% Americans (58.5 million) suffered from physician-diagnosed joint pain and arthritis, and estimated that by 2040, about 26% of US adults (78 million), 18 years or older, will suffer from joint pain and arthritis [4].

Joint pain, inflammation, and injury takes place because of cartilage breakdown, rupture, twisted ligaments, routine wear and tear, as well as abnormal joint mechanics [5-6]. Non-steroidal anti-inflammatory drugs (NSAIDS) and analgesics including acetaminophen can often provide temporary relief but do not provide permanent relief. There are now more than 20 different NSAID drugs around the world exhibiting an array of analgesic, antipyretic and anti-inflammatory properties by inhibiting prostaglandin synthesis [7]. The pro-inflammatory properties of E-series prostanoic acids are amplified through the release of bradykinins, subsequently influencing vascular permeability modulation [8]. "Formation of prostacyclin and thromboxane are inhibited by NSAIDs with pronounced effects on vascular permeability and platelet aggregation [7-8]. Ultimately, the level of prostaglandins is reduced by NSAIDs through the inhibition of cyclooxygenase (COX), the key enzyme necessary for the conversion of arachidonic acid to prostaglandins [9]. Most of the NSAIDs are administered orally, while selected NSAIDS are administered intramuscularly or intravenously to relief acute pain and inflammation [7],[9]. Disease-modifying anti-rheumatic drugs (DMARDs) including methotrexate and hydroxychloroquine, and infliximab, a TNF blocker, demonstrated some efficacy in preventing joint pain and inflammation [10]. However, all these drugs exhibited potential side effects including gastritis and peptic ulcers.

Significant research studies were conducted around the world to assess the efficacy of diverse phytopharmaceuticals and dietary supplements to prevent joint pain and discomforts, and inflammation [5]. The ultimate objective is to maintain healthy joints and prevent the onset of chronic inflammation and ultimately arthritis [6]. An array of in vitro, in vivo, and clinical investigations demonstrated the functional efficacy of diverse nutraceuticals and functional foods in ameliorating joint pain and inflammation. This includes glucosamine hydrochloride, chondroitin, sulfate, N-acetylglucosamine, undenatured type II collagen, hyaluronic acid, proteoglycans, Ananas comosus, Curcuma longa, Boswellia serrata, Zingiber officinale, Radix Tripterygium wilfordii, Mentha spicata, Rosmarinic acid, menthol, green-lipped muscle, and methylsulfonylmethane (MSM), all of which demonstrated broad-spectrum safety and significant efficacy against joint pain and inflammation [5]. In our recent in vitro and in vivo studies, we have demonstrated the efficacy of *Cymbopogon citratus* leaf oil, *Hedychium spicatum* extract, *Zanthoxylum alatum* fruit extract, and Menthol (Mentha arvensis), singly and in combination, in attenuating joint pain and inflammation, as evidenced by their potent inhibitory abilities against TNFα [11].

Ayurvedic botanical herbs and oils are enriched in structurally diverse phytochemicals including antioxidant(s), anti-inflammatory agents, muscle relaxants, and membrane stabilizers [12]. These exhibited versatile health benefits including relief of joint

pain and inflammation without causing any adverse cytotoxic effects. Our previous study and patent [13] exhibited the efficacy of HerboCare or HerboJoint™, prepared using the concept of Ayurveda, in ameliorating joint pain and inflammation in both in vitro and in vivo models [11]. In HerboCare or HerboJoint™ formulation [13], a synergistic patented blend of three essential oils derived from GRAS (Generally Recognized As Safe) affirmed medicinal plants, namely Kattrna (Cymbopogon citratus) leaf oil (3.0%), Sati (*Hedychium spicatum*) extract (1.0%), Tumuru (*Zanthoxylum alatum*) fruit extract (1.0%), and Menthol (Mentha arvensis) (1.3%) were used. It was prepared and delivered in a topical oil-in-water emulsion cream. It was hypothesized that HerboCare or HerboJoint™ cream would ameliorate joint pain and inflammation in human subjects. This clinical investigation explores the efficacy of HerboCare or HerboJoint™ in human volunteers.

MATERIALS AND METHODS

HerboCare or HerboJoint™ cream: As discussed in our previous publication, HerboCare or HerboJoint™ cream was manufactured by Bordoloi Biotech India Pvt. Ltd. (BBIPL) Assam, India, using a combination of essential oils from *Cymbopogon citratus* (CC, 3.0%), *Hedychium spicatum* (HS, 1.0%), *Zanthoxylum alatum* (ZA, 1.0%) in conjunction with USP grade Menthol crystal (M) (1.3 %). Evaluations were conducted, singly and in combination, of these essential oils. Following which, a non-greasy oil-in-water emulsion cream was prepared, which exhibited its efficacy in relieving joint pain and inflammation in pre-clinical studies [Bordoloi and Saini, US Patent, 2019] [13].

Ethical approval: The research design, recruitment procedures were conducted following stringent inclusion and exclusion criteria, clinical procedures, and methodologies. This follows the International Council for Harmonization (ICH) guidelines for Good Clinical Practices as per the formalities of international standards guaranteed by the Declaration of Helsinki and amendments implemented later. Institutional Ethics Committee (IEC) of Govt. Ayurvedic College & Hospital, Jalukbari, Guwahati, Assam, India, approved this study protocol (Protocol #IEC/16/20-136 dated Oct 14, 2016, Ref: IRB Proposal "A Product HerboCare or HerboJoint™, a Herbal Cream Prepared from Essential Oil Prepared by Melet Schloesing Laboratories). Before recruitment, the inclusion and exclusion criteria and IEC-approved study protocol were explained in detail to all subjects and subsequently they were made to sign the consent forms. All recruited subjects read and duly signed the health questionnaire. Subject confidentiality was strictly enforced. All subjects maintained daily diaries of their regular day-to-day activities, which were regularly reviewed and endorsed by study coordinators and principal investigator. Adverse event monitoring was strictly ascertained.

Design of clinical trials: Two sets of clinical trials were conducted over a period of 90 days of treatment. In the first set, a total of 40 patients suffering from joint pain, over a period of 3 to 6 months, were selected. In the second set, a total of 24 subjects were divided into three groups. Group I included 3 healthy individuals using local application of HerboCare or HerboJoint™ cream. Group 2 included 14 patients suffering from joint pain using HerboCare or HerboJoint™ along with other treatments as prescribed by GACH (Government Ayurvedic College and Hospitals), which was an ayurvedic formulation. This Ayurvedic formulation was prepared from the extracts of Mahavatvidhwamsa ras, Khanjankari rasa, Maharasnadi kwath, R-compound, Simhanad guggulu etc. and some Panchakarma therapies like Snehan, Swedan, Matra vasti, Vaitaran vasti etc., exhibiting proven efficacy in the

treatment of joint pain. Group 3 included 3 patients, suffering from joint pain, having treatment prescribed by GACH alone. A total of four subjects dropped out from the study because of personal reasons.

In both sets of trials, it was ensured that the patients were not already on any other treatment and did not use any over the counter (OTC) medicines, at least two weeks prior to the treatment as well as during the entire phase of the treatment. The cream was applied topically over clear, uncut skin around the affected area of the joint thrice a day. The dose of the HerboCare or HerboJoint™ cream for the knee was 1.5 to 2.0 gms or a length of 3-4 cm of the expressed tube. The patients for both the trials were selected on the basis of the following inclusion and exclusion criteria. A list of criteria was also set for withdrawal of patients midway during the trial.

Criteria for inclusion:

A. Age between 35 - 60 years of either sex
B. Presence of any four of the following seven criteria (according 1987, revised criteria of American College of Rheumatology)

1. Morning stiffness: Stiffness in and around joints lasting one hour before maximal improvement (More than 6 weeks duration).
2. Arthritis of three or more joints (at least three joint area, observed by Physician
3. Simultaneously having pain with soft tissue swelling or joint effusion, not just bony overgrowth (More than 6 weeks duration).
4. Arthritis of Hand joints (More than 6 weeks duration).
5. Symmetric arthritis (More than 6 weeks duration).
6. Presence of Rheumatoid Nodules
7. Serum Rheumatoid factor- positive
8. Typical Radiographic changes of arthritis on patient view of hand & wrist radiograph that must include erosions or unequivocal bony decalcification adjacent to involve joints.

Criteria for exclusion:

1. Age below 35 and above 60 years.
2. Patients who develop secondary complication of RA e.g., Pleuro-pericardial disease, severely damaged joint with bed ridden patients.
3. Any other serious illness e.g., Hepatic/ renal failure.
4. Patients diagnosed with other arthritis like Gouty arthritis, tuberculosis arthritis etc.
5. Patients receiving any other medical treatment.

Criteria for withdrawal:

1. Aggravation of the disease during the trial period.
2. Discontinuation of the treatment during trial.
3. Development of any serious complications requiring change in the treatment.

Examination and assessment schedule: Clinical assessment and laboratory investigation were carried out on the 0th day (labelled BT indicating before treatment) and 90th day (labelled AT indicating after treatment) for the first trial. Patients were regularly checked by the attending physicians as per their routine procedures.

For the second trial, samples were collected from patients on the 0th and 90th day of treatment for the three groups, Groups 1, 2 and 3. Blood was collected from the patients, and it was centrifuged to separate the serum. Serum from every individual was collected on days 0, 30, 60 and 90 of treatment and preserved appropriately. TNF-α, a noxious inflammatory marker, was then assessed in these serum samples using ELISA kits. The changes in the subjective and objective

parameters, before and after the treatment, were considered for assessment of efficacy of the HerboCare or HerboJoint™ cream. Samples were analyzed by ELISA in two independent laboratories viz. (a) Institute of Advanced Studies in Science and Technology (IASST), Guwahati, Assam, India, and Indian Institute of Chemical Biology (IICB), Kolkata, West Bengal, India.

Parameters of clinical assessment: Patients were examined for assessment of clinical parameters as recommended by the American Society of Rheumatology [https://www.rheumatology.org/Practice-Quality/Clinical-Support/Clinical-Practice-Guidelines] at specific time intervals as mentioned before and were classified from Grade 0 to Grade III depending on the severity of pain and symptoms (Nil or No pain = 0; Mild = 1; Moderate = 2; Severe = 3). The parameters chosen were joint pain, morning stiffness, tenderness and swelling (the circumference of swollen Proximal inter Phalangeal joints (PIPj) and big/ major joints were measured).

Parameters of functional assessment: Apart from clinical examination of affected areas, functional investigations were also carried out on the 90th day to check the improvement in joint flexibility before and after treatment [28],[29]. This included evaluation of walking time (patients were asked to walk a distance of 150 ft. and time taken was recorded), gripping power (patients were asked to squeeze the inflated cuff up to 50 mm Hg of the sphygmomanometer and the grip power was recorded in mm of mercury upon the rise of mercury column) and pressing power (patient presses the same inflated cuff up to 50 mm Hg against a table.

Measurement of levels of inflammatory cytokines: Serum levels of the pro-inflammatory cytokine TNF-α were evaluated as marker of inflammatory response. The serum was isolated, aliquots were prepared and refrigerated in plastic tubes, and stored at −80°C. The cytokines were then measured with ELISA kit (R & D systems, Minneapolis, MN, USA). The lower limits of detection in each case were set at 1.7 pg/ml (6).

Adverse event monitoring: All subjects were asked to report any adverse events to the study coordinators and principal investigators on a regular basis. Overall, adverse event monitoring was strictly enforced.

Statistical analyses: All data reported were expressed as the means ± standard error (SE) of at least 3 independent replicates for each experiment. Statistical significance between each experimental group (BT and AT) was analyzed using the student's t-test, and a probability value of 0.01 and 0.05 was used as the criterion of significance.

RESULT:

CLINICAL TRIALS IN HUMAN SUBJECTS SUFFERING FROM JOINT PAIN AND INFLAMMATION

Physical assessment of joint pain, set 1: The efficacy of the HerboCare or HerboJoint™ formulation was assessed in clinical settings in patients suffering from chronic joint pain and inflammation. The therapeutic efficacy was investigated after different time spans. The effect of the cream was investigated on three physical attributes namely joint pain, joint swelling, and joint stiffness according to the established protocol of clinical assessment (33) in the 40 randomly chosen patients suffering from joint pain. All the three symptoms were alleviated to a substantial extent owing to the therapeutic efficacy of the formulation (Figure 1). Joint pain reduced by 29% in all patients as it went down from 1.98 + 0.12 (S.E.) to 1.40 + 0.08 (S.E.)(t-value = 5.72, p-

value < 0.0001); Joint swelling was alleviated by 31% in patients as it went down from 0.58 + 0.12 (S.E.) to 0.40 + 0.09 (S.E.)(t-value = 2.87, p-value < 0.006); and Joint stiffness reduced by 60% in patients as it went down from 0.75 + 0.15 (S.E.) to 0.30 + 0.10 (S.E.)(t-value = 4.0, p-value < 0.003). These data were testimonial to the efficacy of the formulation as a reliever of joint pain and inflammation.

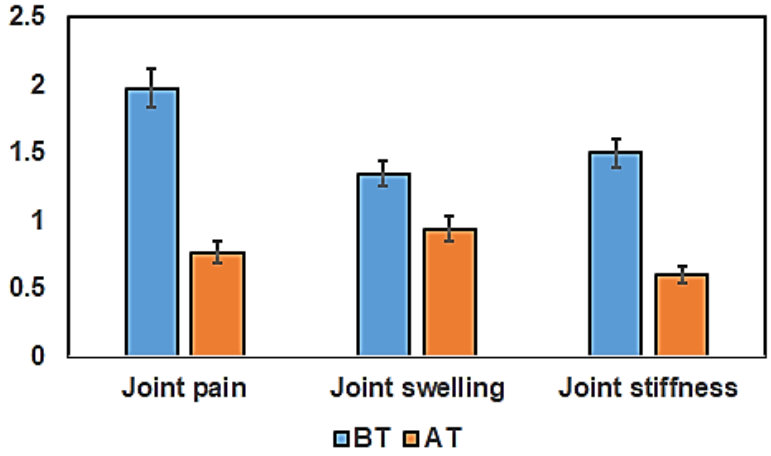


Figure 1: Effect of HerboCare or HerboJoint™ in alleviating joint pain, joint swelling and joint stiffness: In a 90-day investigation, joint pain, Joint Swelling and Joint Stiffness were evaluated in a total of 40 subjects at the initiation and completion of 90 days of treatment. Data are expressed as mean + S.E.

Evaluation of Inflammatory Cytokine TNF-α in affected patients, set 2: We evaluated TNF- α levels in normal and in subjects suffering from OA and RA, which was demonstrated in Figure 2. First, the state of inflammation in the affected patients (n = 17) was determined and compared with that in a generally healthy group of individuals (Control sample size, n = 3). Mean TNF-α level shot up from 117 ±15 (S.E.) pg/ml to 159 ±18 (S.E.) pg/ml at t = 0, indicating that inflammatory response associated with elevated joint pain group of subjects was of a higher grade as compared to that of the Control subjects.

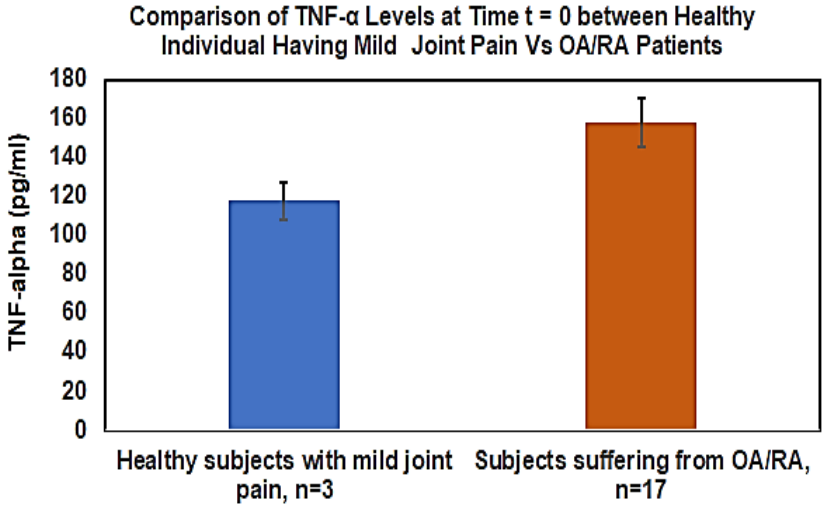


Figure 2: TNF- α levels in healthy subjects (n = 3) and subjects suffering from OA/RA (n = 17) at the Initiation of the study

The status of the inflammatory marker TNF-α was then evaluated in a different set of clinical trials conducted over a period of 90 days (Figure 3). We evaluated TNF- α levels from two different laboratories to exhibit the trend in the reduction. We determined the time-dependent efficacy of HerboCare or HerboJoint™ cream in reducing the TNF-α levels in affected patients the laboratories of IASST (Figure 3). Patients were divided into three groups as elaborated in the Materials and Methods section. In Group-1, patients treated with HerboCare or HerboJoint™ only, the mean TNF-α levels decreased 38% from 117 + 30 (S.E.) pg/ml to 72 + 15 (S.E.) pg/ml. The drop in TNF-α levels was even more significant in Group-2 patients where "GACH" (Government Ayurvedic College and Hospital) prescribed formulation, taken orally due to pain severity, was added along with HerboCare or HerboJoint™ topically, which resulted in a 63% decrease from 169 + 47 (S.E.) pg/ml to 62 + 8 (S.E.) pg/ml. While a small group, Group-3 patients, who were treated only with "GACH", showed a downward trend of TNF-α levels, where it went down 71% from 149 + 57 (S.E.) pg/ml to 44 + 2 (S.E.) pg/ml. This confirmed the role of HerboCare or HerboJoint™ cream in attenuating the heightened inflammatory response.

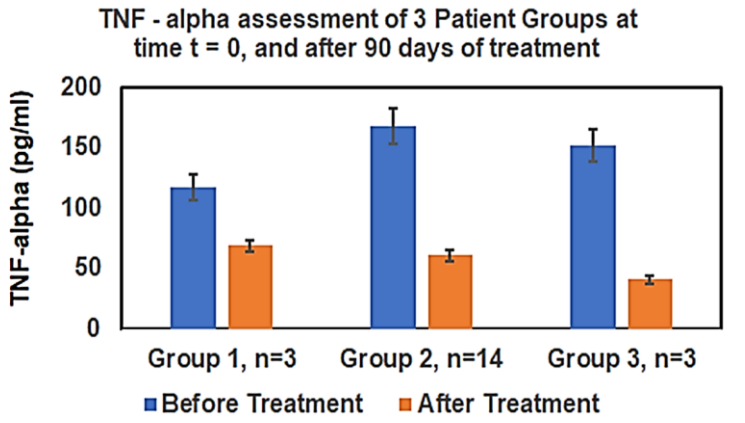


Figure 3: Effects of HerboCare or HerboJoint™ and GACH on TNF-α levels in subjects suffering from joint pain at the initiation (t = 0) and completion of the study (t = 90 days): ELISA Data at Institute IASST: Comparative Assessment of TNF-α levels in the Three Patient Groups: Group 1 - Treated with HerboCare or HerboJoint™ only (n = 3); Group 2- Treated with HerboCare or HerboJoint™ Cream and GACH Prescribed Formulation, Taken Orally (n = 14); Group 3 – Treated with GACH Prescribed Formulation Alone (n = 3).

In a second parallel study of shorter duration, time dependent efficacy of HerboCare or HerboJoint™ cream and GACH treatment, singly and in combination, on the changes in TNF-α levels in the laboratories of IICB (Figure 4). The time-dependent efficacy of HerboCare or HerboJoint™ cream and a combination of HerboCare or HerboJoint™ cream and GACH treatment was assessed on the reduction of TNF-α levels. A time-dependent decline in TNF-α levels from 57 + 3 pg/ml (baseline) to 54 + 7 pg/ml at 30-days of treatment with HerboCare or HerboJoint™ was observed, which was further reduced to 50 + 12 pg/ml at 60-days of treatment. In the combination HerboCare or HerboJoint™ and GACH study group, time-dependent efficacy of HerboCare or HerboJoint™ along with the other treatment prescribed by GACH was determined on subjects suffering from joint pain. A decline in TNF-α levels from 53 + 14 pg/ml (baseline) to 45 + 11 pg/ml was observed at 30 days of treatment, which was further reduced to 33 + 20 pg/ml at 60 days of treatment. These declines showed a

consistent trend in lowering of the TNF-α.

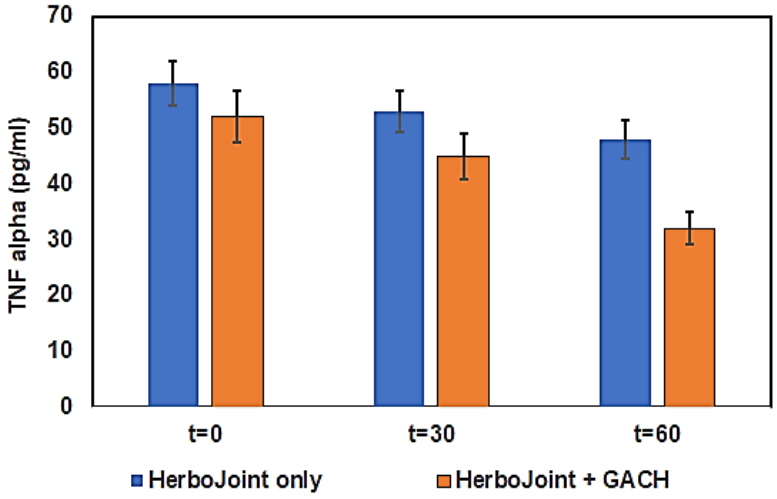


Figure 4: Effects of HerboCare or HerboJoint™ and GACH on TNF-α levels in subjects suffering from joint pain at the Initiation (t = 0), Mid-Point (t = 30), and completion of the study (t = 60 days): ELISA Data at Institute IICB: comparative assessment of TNF-α levels in human blood plasma in two patient groups at 0, 30 and 60 days of treatment: Group 1 - treated with HerboCare or HerboJoint™ only; Group 2- treated with HerboCare or HerboJoint™ Cream and GACH prescribed formulation, taken orally.

DISCUSSION

Inflammatory terminologies including joint pain, joint and muscle stiffness, swollen joints, and reddening of the joints were first described using a proper scientific terminology in 1611 by a French physician, Dr. Guillaume de Baillou [14]. These inflammatory sequelae lead to various musculoskeletal illnesses and a range of maladies. If these initial joint pain and inflammation can't be controlled then it is aggravated to diverse chronic inflammatory disorders including gout, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and fibromyalgia [15-16]. Overall, these diseases lead to significant functional limitation and physical disability [17]. In fact, prevalence of joint pain and inflammation increases remarkably with advancing age, while lifestyle factors including obesity and sedentary lifestyle have pronounced influence in escalating joint pain and inflammatory responses [17-18].

Advancing age is involved in reducing the tensile strength and enhancing the stiffness of the joints, especially the articular cartilages [17],[19],[20]. A cascade of molecular events occurs, involving an increase in hyaluronan content, a rise in the prevalence of cartilage calcification, along with degradation and loss of joint type II collagen. Additionally, age-related changes take place in the chondrocytes [19-21]. These in turn cause a reduction in anabolic response to IGF-1 stimulation along with an enhanced production of endogenous oxygen free radicals leading to decreased responsiveness to growth factor (21-23). All these modifications in the chondrocytes function cause a reduced capacity to repair the damaged articular matrix (24). Thus, a reduction in the proliferative capacity of chondrocytes with advancing age leads to the apoptosis of the chondrocytes.

Several other factors including (a) obesity and overweight, (b) mechanical stress and cartilage loss, and (c) genetics and cartilage loss induce significant inflammatory responses in causing joint degradation [25]. This involves the stimulation of IGF-1 and TGF-β1 synthesis in chondrocytes. Moreover, chondrocytes in conjunction with IL-1 and leptin enhance the production of nitric oxide [25-26]. Overall, this massive imbalance enhances the production of inflammatory processes. Studies have also shown the involvement of IL-6 and C-reactive protein in this pathogenesis. During mechanical stress, COX-2 plays a pronounced role in enhanced production of oxidative stress and apoptosis. Genetic impairment has exhibited a significant role in this pathogenesis [26-28].

Efforts have been made to prevent joint pain and inflammation using structurally diverse pharmaceuticals and synthetic drugs, which have demonstrated temporary benefits in alleviating joint pain and inflammation, however, no long-term solution was achieved. Several clinical studies were conducted on these numerous drugs and pharmaceuticals [29]. Also, these drugs are associated with multiple side effects. Moreover, exorbitant cost and associated toxic manifestations of structurally diverse NSAIDS, methotrexate and pain-relieving drugs limited their use [29-31]. On the other hand, various nutraceuticals and standardized botanical extracts, including glucosamine hydrochloride, chondroitin sulfate, N-acetylglucosamine, undenatured type II collagen, hyaluronic acid, proteoglycans, standardized Boswellia serrata extracts such as 5-Loxin and Apresflex, turmeric root extract, Zingiber officinale root extract, Mentha spicata extract, Radix Tripterygium wilfordii extract, Ananas comosus extract, Green-Lipped mussel, and other marine-based nutraceuticals, methylsulfonylmethane (MSM), and dehydroepiandrosterone, have demonstrated potent beneficial effects against joint pain, discomfort, and inflammation without causing significant adverse effects. Moreover, many of these botanicals exhibit potent anti-carcinogenic, hepatoprotective, antidiabetic and anti-viral activities [5,32]. In the present investigation, we have exhibited the efficacy of standardized *Cymbopogon citratus* leaf oil, *Hedychium spicatum* extract, *Zanthoxylum alatum* fruit extract, and Menthol (Mentha arvensis), singly and in combination, in attenuating joint pain and inflammation, as evidenced by their potent inhibitory abilities against TNFα. Furthermore, in this investigation, we have demonstrated their combined synergistic efficacy in human volunteers suffering from joint pain and inflammation.

It may be mentioned that the USFDA has the final say on whether it will add or remove any over the counter (OTC) formulation from the GREASE designation – short for “generally recognized as safe and effective” [33]. Essential oils used in Ayurveda and in the current study were rated as GRAS, for which the current study was undertaken to demonstrate their effectiveness[34].

CONCLUSION:

HerboCare or HerboJoint™, formulated with Generally Recognized as Safe (GRAS)-affirmed botanical ingredients, demonstrates clinical effectiveness in relieving joint pain and inflammation. This Ayurvedic approach offers a safe and cost-effective alternative for managing these debilitating conditions. The entire process of evaluation Paper-I and Paper-II are captured as shown in Figure 5.

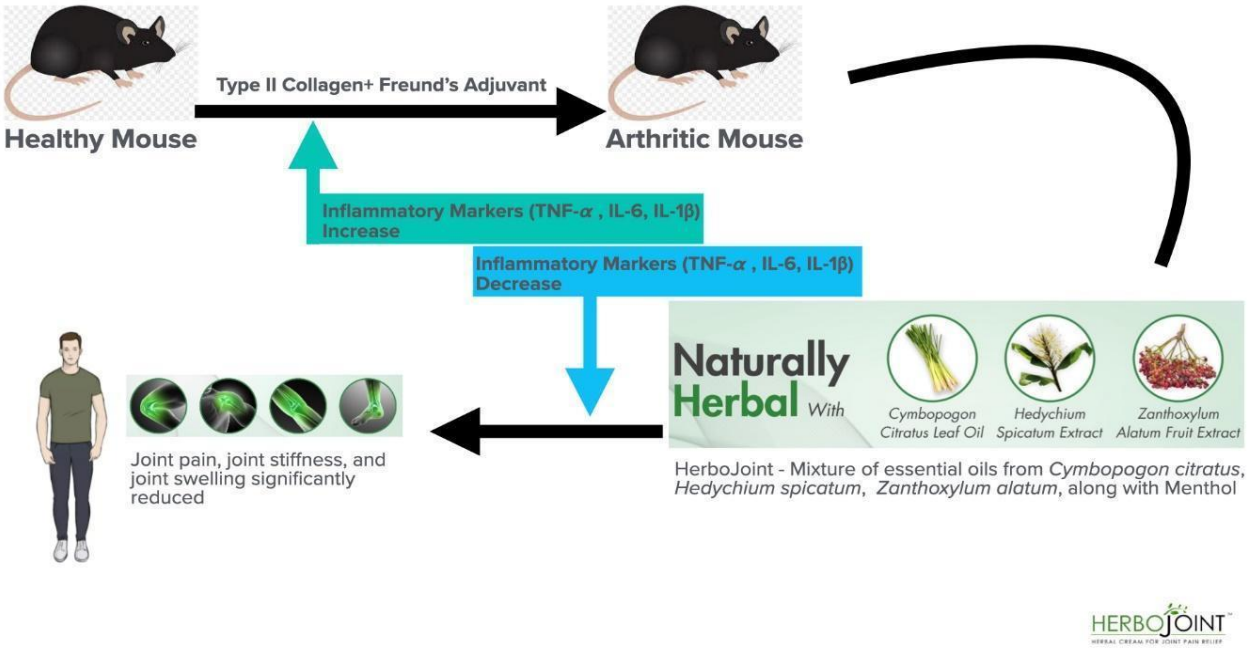


Figure 5: A scientific flowchart capturing the concept of pre-clinical to clinical investigation on the HerboCare or HerboJoint™ topical cream.

List of abbreviations: IICB: Indian Institute of Chemical Biology**;** NIPER: National Institute of Pharmaceutical Education and Research**;** CSIR: Council of Scientific & Industrial Research**;** CC: *Cymbopogon citratus***;** HS: *Hedychium spicatum***;** ZA: *Zanthoxylum alatum***;** RA: Rheumatoid Arthritis**;** OA: Osteoarthritis; DMARDs: Disease-Modifying Anti-Rheumatic Drugs**;** NSAIDs: Non-Steroidal Anti-Inflammatory Agents, GRAS: Generally Recognized as Safe

Competing interests: Authors listed under Bordoloi Biotech LLC were responsible for preparing the Ayurvedic oils' emulsion cream samples. The rest of the authors carrying out the pre-clinical studies declare that they had no competing or financial interests.

Acknowledgements: We wanted to acknowledge a few names for helping us in various capacities in this endeavor. Dr. Arun Bandyopadhyay, Director (CSIR-IICB, WB), and specifically Dr. Parasuraman Jaisankar (CSIR-IICB, WB), Dr. Narayan C. Talukdar, Director (IASST, Guwahati, India), Dr. Pranab K. Boruah, MD, ex-Medical Doctor of the (Clinical Center-NEIST Assam), and Dr. Prodeep Phukan, professor of chemistry (Guwahati University, India). Finally, we wanted to thank Niten Barman, associate professor (GACH, Assam, India) for his guidance in the statistical analysis of the clinical data.

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