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AflaB2® and osteoarthritis: a multicentric, observational, post-marketing surveillance study in Indian patients suffering from knee osteoarthritis

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ABSTRACT

Background: Osteoarthritis (OA) is one of the most debilitating chronic degenerative joint disorder characterized by pain, inflammation and stiffness of joints with wear and tear of the cartilage. Recent evidences suggest the involvement of the immune pathway in OA development. This study was conducted to evaluate the efficacy and safety of AflaB2® capsules containing Aflapin® and native collagen type II in knee OA patients.
Methods: Total 40 knee OA subjects were enrolled at the out-patient department (OPD) of three different sites under supervision of physicians as per the inclusion and exclusion criteria of the study. Subjects were instructed to consume AflaB2® capsules once daily orally for three months. They were informed to visit the respective study center as per the schedule visits to assess and record the efficacy and safety.
Results: AflaB2® treatment showed significant reduction in pain and stiffness with improvement in physical functions compared to the baseline. The reduction in pain score was observed from 2nd visit on visual analogue scale (VAS). The VAS score was reduced to 1.63±1.23 ($p<0.001$) from its baseline score 6.0±1.04 at the end of the treatment. The WOMAC Total Score was reduced to 18.1±6.04 ($p<0.001$) from its baseline score 74.4±8.07 at the end of the treatment. The improvement was observed in WOMAC pain, stiffness and physical functions score. No significant side-effect was reported with AflaB2® treatment throughout the study.
Conclusions: The present study provides the evidence in support of the potential efficacy and excellent tolerability of oral intake of AflaB2® capsules in reducing OA symptoms.

Keywords: Aflapin®, Native collagen type II, AflaB2®, Osteoarthritis, Oral tolerization, 5-LOX

INTRODUCTION

Osteoarthritis (OA) is a common chronic, degenerative joint disorder and the foremost cause of pain and disability among adults.[1] Joint tissue changes related to aging can contribute to the development of osteoarthritis. Reduction in joint mobility, increased joint stiffness and joint pain are characteristic clinical symptoms of osteoarthritis.[2] The knees, hips and hands are the most commonly affected joints in OA. OA pathophysiology affects the whole joint which is characterized by cartilage degradation, bone remodelling, osteophyte formation and synovial inflammation leading to pain, stiffness, swelling and loss of normal joint functions.[3] OA is a considerable burden in both social and economic aspects. The most common risk factors of OA are age, joint instability, obesity, peripheral neuropathies, muscle weakness and crystal deposition disease of the joints.[4] OA is a complex disorder, its initiation, progression and severity may be influenced by multiple factors. Osteoarthritis involves cartilage breakdown which may be due to joint trauma or overuse, tissue damage etc.[5] Two important pathways in the

pathogenesis of osteoarthritis are the mechanical pathway and the immune pathway. Mechanical injuries like trauma, mechanical stress, joint overuse, tissue damage, etc. are contributing to the mechanical pathway of OA development.[5] The immune pathway for OA development defines the response of our immune system against antigenic components produced from cartilage breakdown products. T-cell induced synovial membrane inflammation is reported in at least 50% of OA patients suggesting the role of T-cells as a contributor to the immunological pathway of osteoarthritis.[6]

A therapeutic intervention addressing the mechanical and immune pathways can be a comprehensive approach for OA management. Current OA management includes non-steroidal anti-inflammatory drugs (NSAIDs), analgesics like tramadol & paracetamol as well as disease modifying molecules. However, their efficacy in controlling the immune mediated OA progression is not established.

Aflapin® is a patented and most selective 5-LOX enzyme inhibitor developed by Laila nutraceuticals research and development center, India. Aflapin® is a novel synergistic composition of Boswellia serrata extract selectively enriched with acetyl-11-keto-β-boswellic acid (AKBA) and B. serrata non-volatile oil.[7] It provides pleiotropic benefits acting on multiple cellular events of OA.[8] Aflapin® has anti-inflammatory, cartilage-protective and anti-osteoarthritic effect exhibiting better bioavailability compared to other *B. serrata* extracts commercially available in the market.[8,9]

Native collagen type II is a proprietary ingredient developed by Bioiberica, S.A.U, Spain. It acts by suppressing immune mediated cartilage degradation by a unique mechanism of "Oral Tolerization" to reduce joint pain and inflammation.[10,11] However, the clinical evidence of a combination of Aflapin® and native collagen type II on Indian population is lacking.

This study aims to evaluate the efficacy and safety of a combination of Aflapin® and native collagen type II as AflaB2® capsules in the Indian population suffering from osteoarthritis.

METHODS

Study design

This was a pilot, observational, single arm, multicentric open label real-world evidence study.

Study duration

This study was conducted between June'19 to March'20 at three sites viz. Elite Bone Care and Gynae Cosmetic Surgery center, Delhi, Shree Mahaveer Ortho Clinic, Delhi and Radiance Clinics, Chennai.

Inclusion criteria

The inclusion criteria for the subjects were; subjects must understand risk and benefits of protocol and must be able to give informed consent, subjects aged 40 to 80 years, females of childbearing potential must agree to use an approved form of birth control and to have a negative pregnancy test results, subjects with unilateral and bilateral OA of the knee for more than 3 months, subjects with VAS score during the most painful knee movement between 40 and 70 mm after 7 days of withdrawal of usual medication, subjects must be able to walk and subjects must be available during the entire study period.

Exclusion criteria

The exclusion criteria for the subjects were; history of underlying inflammatory arthropathy or severe rheumatoid arthritis, hyperuricaemia (>440 µmol/L) and/or history of gout, recent injury in the area affected by the OA of knee and expectation of surgery in the next four months, subjects who received Intraarticular corticosteroid injection in preceding 3 months, hypersensitivity to NSAIDs, abnormal liver or kidney function tests, history of peptic ulceration and upper GI haemorrhage, congestive heart failure, hypertension, cancer, hyperkalaemia, subjects with major abnormal findings on CBC, history of coagulopathy, haematological or neurological disorders, subjects with high alcohol intake (>2 standard drinks per day), subjects taking NSAIDs for less than 3 weeks before initiation of the study and subjects with BMI >30 kg/m^2.

Study treatments

The nutraceutical composition was provided as AflaB2® capsules (Sundyota Numandis Pharmaceuticals Pvt Ltd., Ahmedabad, India). Each capsule contained 100 mg Aflapin® and 40 mg native collagen type II. Subjects were informed to consume orally one capsule per day for the period of three months and visit the study center on day 0, day 5, day 15, day 30, day 60 and day 90 to assess the safety and efficacy parameters as per the protocol. All the subjects were advised to consume NSAIDs/analgesics as a rescue medicine as required.

Methodology

The subjects diagnosed with knee OA and meeting inclusion/exclusion criteria were considered eligible for the study. Subjects were informed by the physician to ingest AflaB2® capsules as per the study protocol. For each subject, a predesigned case record form (CRF) was maintained wherein demographic profile and results from evaluation parameters were recorded. Subjects visited study center on day 0, day 5, day 15, day 30, day 60 and day 90 for evaluation of efficacy and safety of treatment based on the visual analogue scale (VAS) and Western Ontario and McMaster universities arthritis index (WOMAC) efficacy rating scales. Subjects were asked to report any adverse events during intervention period.

Statistical analysis

Statistical analyses were performed using GraphPad prism 8 software (Graph Pad software, USA). Differences among the results recorded on various visits were analysed using one-way ANOVA, followed by Dunnett's multiple comparison test as a post-hoc analysis. Statistical significance was considered at ****p<0.0001; ***p<0.001; **p<0.01; *p<0.05 when compared with the results recorded at baseline (day 0).

RESULTS

Forty subjects were included in the study with the mean age 58.95±8.30 years. 23 female subjects with mean age 58.7±7.51 years and 17 male subjects with mean age 59.29±9.5 years, completed the study.

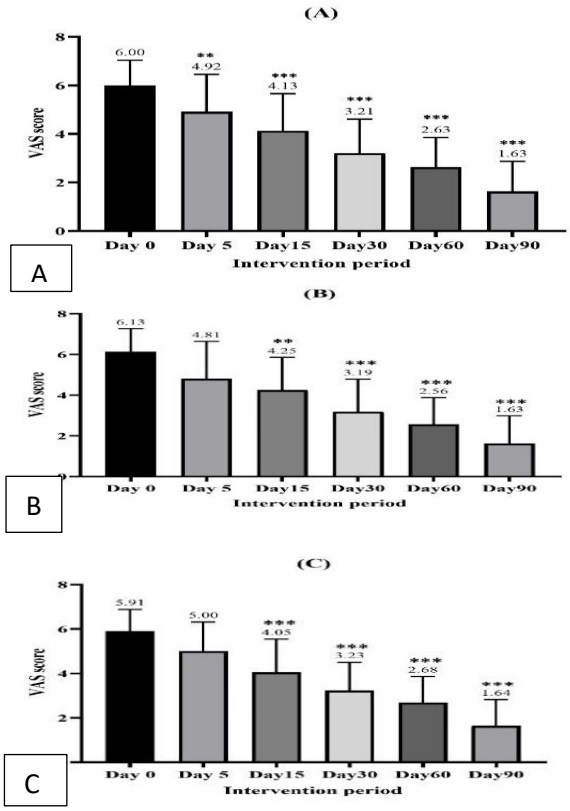

Figure 1: Effect of the treatment on the VAS score of the subjects as recorded on various visits. (A) VAS score of all subjects, (B) VAS score of male subjects, (C) VAS score of female subjects. Values are expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA, followed by Dunnett's multiple comparison test as a post-hoc analysis to identify significant differences among the visits. ***p<0.001; **p<0.01, when compared with the scores on day 0.

Effect of the treatment on VAS score

AflaB2® treatment showed significant reduction on pain assessed in VAS scale in all subjects (A). Gender based analysis showed significant improvement in pain in males and females (B and C). Baseline VAS score was reported as 6.0±1.04. The VAS score was reduced to 4.92±1.53, 4.13±1.52, 3.21±1.39, 2.63±1.21 and 1.63±1.23 on day 5, day 15, day 30, day 60 and day 90 respectively indicated progressive reduction in pain (Table 1 and Figure 1).

Effect of the treatment on WOMAC score

Treatment with AflaB2® showed significant and progressive reduction in OA symptoms assessed on WOMAC score. The significant reduction in WOMAC total score, WOMAC pain score, WOMAC stiffness score and WOMAC physical functions score was reported with AflaB2® treatment on day 5, day 15, day 60 and day 90 compared to the baseline (Table 1 and Figure 2).

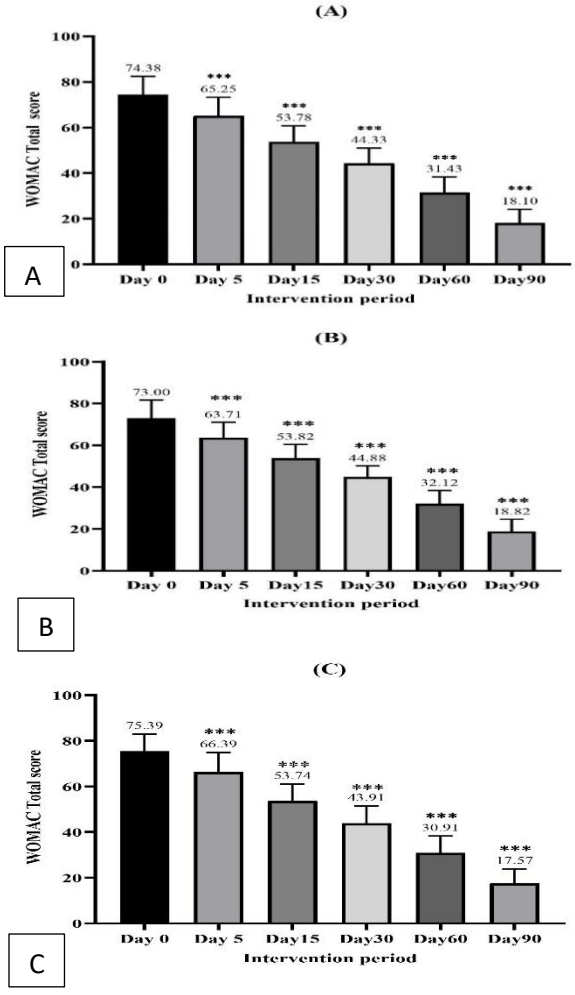

Figure 2: Effect of the treatment on WOMAC total scores of the subjects as recorded on various visits. (A) WOMAC total score of all subjects, (B) WOMAC total score of male subjects, (C) WOMAC total score of female subjects. Values are expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA, followed by Dunnett's multiple comparison test as a post-hoc analysis to identify significant differences among the visits. ***p<0.001; **p<0.01, when compared with scores on day 0.

Table 1: Effect of AflaB2®treatment on VAS and WOMAC subscale.

Scale	Day 0	Day 5	Day 15	Day 30	Day 60	Day 90
VAS Score	6.0±1.04	4.92±1.53**	4.13±1.52***	3.21±1.39***	2.63±1.21***	1.63±1.23***
WOMAC pain score	14.83±2.52	13.13±2.71**	10.78±2.22***	9.2±1.96***	6.42±2.14***	3.35±1.79***
WOMAC stiffness score	5.35±2.05	4.77±2.03	4.07±1.76**	3.37±1.69***	2.52±1.37***	1.51±0.99***
WOMAC physical function score	54.2±5.99	47.35±5.43***	38.8±4.32***	31.75±4.08***	22.45±4.57***	13.38±4.37***
WOMAC total score	74.4±8.07	65.25±8.06***	53.78±6.98***	44.33±6.64***	31.43±6.89***	18.1±6.04***

Values are expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA, followed by Dunnett's multiple comparison test as a post-hoc analysis to identify significant differences among the visits. ***p<0.001; **p<0.01, when compared with scores on day 0.

Tolerability

No significant adverse event was reported throughout the study period reiterating that the AflaB2® treatment was well tolerated and safe.

DISCUSSION

Clinical evidence of Aflapin® and native collagen type II in the management of osteoarthritis have been examined in several clinical studies as an individual ingredient. OA affects entire joint structures including articular cartilage, synovial membrane, subchondral bone as well as ligaments. During the progression of OA, the extracellular matrix (ECM) of cartilage is actively remodelled by chondrocytes under inflammatory conditions.[12] OA is a complex and chronic disorder. The exact pathophysiology of OA is not understood completely till date. However, the mechanical pathway and immune pathway are known to contribute to the development of OA. Mechanical injuries of various aetiology are associated with cartilage breakdown process. The inflammatory process is also accompanied with cartilage breakdown process. 5-lipoxygenase (5-LOX) enzyme has an important role in the pathogenesis of osteoarthritis.[13] Increased activity of 5-LOX enzyme is associated with the release of various inflammatory cytokines like IL-1β[14], TNF-α[14] and ICAM[15] leads to the triggering of inflammation in osteoarthritis. Additionally, 5-LOX enzyme stimulates the activity of matrix metalloproteinases (MMPs) which trigger cartilage breakdown.[16] Cartilage breakdown products (components) have antigenic properties which stimulate the immune pathway.[5]

Immune mediated progression of osteoarthritis occurs when chondrocytes act as "Professional" Antigen Presenting Cells when they come into direct contact with T cells. Immune system may induce autoimmune arthritis when cartilage components are exposed to it. This leads to initiation of synthesis of proinflammatory cytokines, chemokines, prostaglandins, nitric oxide as well as destructive enzymes resulting into the destruction of cartilage matrix. Subsequent exposure to cartilage antigens to the immune system results into autoimmune reaction to the cartilage in OA. Such autoimmune reactions keep on destroying cartilage with releasing more autoantigens resulting into chronic inflammation and injuries to the articular cartilage. Chondrocytes have the ability to phagocytize collagen fragments and to process exogenous antigens.[5]

Aflapin® is a potent selective 5-LOX enzyme inhibitor exhibiting significant anti-inflammatory and analgesic properties by inhibiting various proinflammatory cytokines like IL-1 β, TNF- α, ICAM, etc.[8] It provides cartilage protective effect by inhibiting cartilage degrading MMP enzymes.[17] In vitro studies have reported an ability of Aflapin® to stimulate the synthesis of glycosaminoglycans (GAG) suggesting its anabolic effect on cartilage health.[8] Aflapin® has a better bioavailability compared to other *Boswellia serrata* extracts due to the presence of non-volatile oil fraction. This non-volatile oil fraction present in Aflapin® might be acting as a vehicle to provide the basis for more bioavailable AKBA in systemic circulation and to reach to the target cells.[8] A randomized double-blind placebo controlled study has reported the ability of Aflapin® in reducing joint pain, joint stiffness and physical functions assessed using VAS, WOMAC and Lequesne functional index (LFI) score significantly higher than other *Boswellia serrata* extract as well as placebo.[8] In another randomized, double-blind placebo controlled study, Aflapin® treated group reported clinically and statistically significant improvements in pain scores and physical function scores compared to the placebo group assessed by VAS, WOMAC and LFI scores. Aflapin® treated group showed significant pain reduction in as early as 5 days of treatment, thus indicating its potent pain-relieving ability.[7] Safety and tolerability of Aflapin® is established on biochemical, haematological and urine parameters through a clinical study.[7] *B. serrata* extract in

combination with Curcuma longa extract and Sesame oil was reported as fast acting and safe alternative for acute pain relief comparable to paracetamol.[18] *B. serrata* was also reported to reduce the need of rescue analgesics in addition to modulating inflammatory cytokines in subjects suffering from knee osteoarthritis.[19] A Combination of *B. serrata* extract with bromelain showed improvement in conducting daily activities suggesting its role in improving quality of life (QoL) in OA patients.[20] *B. serrata* extract is reported to improve the knee joint gap and to reduce osteophytes on radiological parameters in OA subjects. It also reduces serum C-reactive protein (CRP).[21] *Boswellia serrata* extract supplementation improves recovery after ankle sprain to relieve trauma associated with sports activities with reduced need of rescue medicines.[22] *Boswellia serrata* extract in combination with Curcuma longa extract has shown comparable efficacy with ibuprofen or diclofenac in knee OA patients.[23] Aflapin® is considered amongst the top 10 nutraceuticals used for OA in India.[24] The No Observed Adverse Effect Level (NOAEL) of Aflapin® is found to be greater than 2500 mg/kg body weight indicating its broad-spectrum safety.[9]

Native collagen type II provides protection against immune mediated cartilage degradation by a unique mechanism of oral tolerization. Orally ingested native collagen type II antigens interact with Peyer's patches in the gut associated lymphoid tissue, resulting in turning off the T-cell attack to the structural protein collagen type II in the cartilage. This mechanism of action of native collagen type II is responsible for its cartilage protection as well as anti-inflammatory effects in OA.[25] In a randomized, double-blind comparative controlled study, the addition of native collagen type II with paracetamol showed a significant reduction in WOMAC pain score, WOMAC stiffness score, WOMAC physical function score, WOMAC total score and reduction in VAS score compared to the group which received only paracetamol. Native collagen type II treated group showed a significant reduction in the level of cartilage degradation biomarkers like Coll2-1 NO2, Coll2-1 and fibulin-3 confirming its ability in preventing immune mediated cartilage degradation.[25] An observational, retrospective study of native collagen type II has reported a reduction in uCTX-II as well as radiological scores in individuals suffering from hand erosive OA indicating its potential to slow down the progression of OA. Native collagen type II treatment also leads to a significant reduction in the progression of bone decay rate.[11]

In the present study, we observed the efficacy of AflaB2® capsules on VAS and WOMAC scale indicating its potential role in reducing OA symptoms like pain, inflammation and joint stiffness. The possible explanation for these effects might be the dual action of a combination of Aflapin® and native collagen type II. Aflapin® acts on the mechanical pathway while native collagen type II acts on the immune mediated pathway of OA to control cartilage degradation as well as to control symptoms of OA.

CONCLUSION

The present study provides the evidence in support of the potential efficacy and excellent tolerability of oral intake of AflaB2® capsules in reducing OA symptoms.

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Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Roemer FW, Demehri S, Omoumi P, Link TM, Kijowski R, Saarakkala S et al. State of the Art: Imaging of Osteoarthritis-Revisited 2020. Radiol. 2020;296(1):5-21
2. Chow YY, Chin KY. The Role of Inflammation in the Pathogenesis of Osteoarthritis. Mediators Inflamm. 2020; 2020:8293921.
3. Primorac D, Molnar M, Rod E, Jeleč Z, Čukelj F, Matišić V et al. Knee Osteoarthritis: A Review of Pathogenesis and State-Of-The-Art Non-Operative Therapeutic Considerations. Genes (Basel). 2020;11(8):854.
4. Kolasinski SL, Neogi T, Hochberg MC, Oatis C, Guyatt G, Block J et al. 2019 American College of Rheumatology/Arthritis Foundation Guideline for the Management of Osteoarthritis of the Hand, Hip, and Knee. Arthritis Care Res (Hoboken). 2020;72(2):149-62.
5. Sokolove J, Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. Ther Adv Musculoskeletal Dis. 2013;5(2):77-94.
6. Yuan GH, Masuko-Hongo K, Kato T, Nishioka K. Immunologic intervention in the pathogenesis of osteoarthritis. Arthritis Rheum. 2003;48(3):602-11.
7. Vishal AA, Mishra A, Raychaudhuri SP. A double blind, randomized, placebo controlled clinical study evaluates the early efficacy of aflapin in subjects with osteoarthritis of knee. Int J Med Sci. 2011;8(7):615-22.
8. Sengupta K, Kolla JN, Krishnaraju AV, Yalamanchili N, Rao CV, Golakoti T et al. Cellular and molecular mechanisms of anti-inflammatory effect of Aflapin: a novel Boswellia serrata extract. Mol Cell Biochem. 2011;354(1-2):189-97.
9. Krishnaraju AV, Sundararaju D, Vamsikrishna U, Suryachandra R, Machiraju G, Sengupta K et al. Safety and toxicological evaluation of Aflapin: a novel Boswellia-derived anti-inflammatory product. Toxicol Mech Methods. 2010;20(9):556-63.
10. Park KS, Park MJ, Cho ML, Kwok SK, Ju JH, Ko HJ et al. Type II collagen oral tolerance; mechanism and role in collagen-induced arthritis and rheumatoid arthritis. Mod Rheumatol. 2009;19(6):581-9.
11. Scarpellini M, Lurati A, Vignati G, Marrazza MG, Telese F, Re K et al. Biomarkers, type II collagen, glucosamine and chondroitin sulfate in osteoarthritis

follow-up: the "Magenta osteoarthritis study". J Orthop Traumatol. 2008;9(2):81-7.
12. Maldonado M, Nam J. The role of changes in extracellular matrix of cartilage in the presence of inflammation on the pathology of osteoarthritis. Biomed Res Int. 2013;2013:284873.
13. Attur M, Dave M, Abramson SB, Amin A. Activation of diverse eicosanoid pathways in osteoarthritic cartilage: a lipidomic and genomic analysis. Bull NYU Hosp Jt Dis. 2012;70(2):99-108.
14. He Wendy, Pelletier JP, Martel-Pelletier J, Laufer S, Battista JAD. Synthesis of interleukin 1beta, tumor necrosis factor-alpha, and interstitial collagenase (MMP-1) is eicosanoid dependent in human osteoarthritis synovial membrane explants: interactions with anti-inflammatory cytokines. J Rheumatol. 2002;29(3):546-53.
15. Kurokouchi K, Kambe F, Yasukawa K, Izumi R, Ishiguro N, Iwata H et al. TNF-alpha increases expression of IL-6 and ICAM-1 genes through activation of NF-kappaB in osteoblast-like ROS17/2.8 cells. J Bone Miner Res. 1998;13(8):1290-9.
16. Martel-Pelletier J, Wildi LM, Pelletier JP. Future therapeutics for osteoarthritis. Bone. 2012;51(2):297-311.
17. Sengupta K, Krishnaraju AV, Vishal AA, Mishra A, Trimurtulu G, Sarma KVS et al. Comparative efficacy and tolerability of 5-Loxin and Aflapin Against osteoarthritis of the knee: a double blind, randomized, placebo controlled clinical study. Int J Med Sci. 2010;7(6):366-77.
18. Rudrappa GH, Chakravarthi PT, Benny IR. Efficacy of high-dissolution turmeric-sesame formulation for pain relief in adult subjects with acute musculoskeletal pain compared to acetaminophen: A randomized controlled study. Med (Baltimore). 2020;99(28):e20373.
19. Kulkarni PD, Damle ND, Singh S, Yadav KS, Ghante MR, Bhaskar VH et al. Double-blind trial of solid lipid Boswellia serrata particles (SLBSP) vs. standardized Boswellia serrata gum extract (BSE) for osteoarthritis of knee. Drug Metabol Pers Ther. 2020;2020:104.
20. Italiano G, Raimondo M, Giannetti G, Gargiulo A. Benefits of a Food Supplement Containing Boswellia serrata and Bromelain for Improving the Quality of Life in Patients with Osteoarthritis: A Pilot Study. J Altern Complement Med. 2020;26(2):123-9.
21. Majeed M, Majeed S, Narayanan NK, Nagabhushanam K. A pilot, randomized, double-blind, placebo-controlled trial to assess the safety and efficacy of a novel Boswellia serrata extract in the management of osteoarthritis of the knee. Phytother Res. 2019;33(5):1457-68.
22. Feragalli B, Ippolito E, Dugall M, Cacchio M, Belcaro G, Cesarone MR et al. Effectiveness of a novel boswellic acids delivery form (Casperome®) in the management of grade II ankle sprains due to sport trauma - a registry study. Eur Rev Med Pharmacol Sci. 2017;21(20):4726-32.
23. Pinzon RT, Wijaya VO. Curcuma longa and Boswellia Serrata for improving functional status in osteoarthritis patients: From bench to bedside evidences. Asian J Med Sci. 2019;10(5):1-5.
24. Vaishya R, Agarwal AK, Shah A, Vijay V, Vaish A. Current status of top 10 nutraceuticals used for Knee Osteoarthritis in India. J Clin Orthop Trauma. 2018;9(4):338-48.
25. Bakilan F, Armagan O, Ozgen M, Tascioglu F, Bolluk O, Alatas O. Effects of Native Type II Collagen Treatment on Knee Osteoarthritis: A Randomized Controlled Trial. Eurasian J Med. 2016;48(2):95-101.

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