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Research Article

Experimental Evaluation of Anti-arthritic Activity of OstoCan-V50 Tablets Using Monosodium Iodoacetate-Induced Osteoarthritis in Wistar Rats

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Abstract

Background: Osteoarthritis (OA), a prevalent degenerative joint disorder, causes significant disability due to cartilage degradation, inflammation, and pain. Current treatments offer symptomatic relief but fail to modify disease progression and carry adverse effects. OstoCan-V50, a novel polyherbal formulation containing *Withania somnifera (L.)*, *Boswellia serrata Roxb. ex Coleb., Cissus quadrangularis (L.)*, *Kukkudandatwak Bhasma*, and *Cannabis sativa (L.)*, holds potential as a safer, disease-modifying therapy. This study evaluates its anti-arthritic efficacy and safety in a preclinical model. Methods: Following the Animal Research: Reporting of In Vivo Experiments, Version 2.0 (ARRIVE 2.0) and Committee for the Control and Supervision of Experiments on Animals (CCSEA) guidelines, female Wistar rats with monosodium iodoacetate-induced osteoarthritis were divided into six groups (n=8), including controls, diclofenac (10 mg/kg), and OstoCan-V50 (12.87, 25.75, 51.5 mg/kg). Treatments were given orally for 28 days. Knee thickness, pain threshold, serum cytokines, haematology, radiology, and histopathology were assessed. Acute toxicity (2000 mg/kg) followed OECD Organisation for Economic Co-operation and Development Guideline 423(OECD). Data were analysed using ANOVA ($p < 0.05$). Results: OstoCan-V50 significantly reduced knee joint inflammation, increased pain threshold, and improved food intake, water intake, and body weight in MIA-induced osteoarthritis. Serum TNF-α and IL-1β levels decreased markedly ($p < 0.0001$), with the 51.5 mg/kg dose showing efficacy comparable to diclofenac. Haematological alterations were normalized, while radiology and histopathology demonstrated reduced joint damage and greater viable chondrocyte counts. No toxicity was observed at 2000 mg/kg in the acute study. Conclusion: OstoCan-V50 demonstrated significant anti-arthritic, anti-inflammatory, and analgesic effects with a safety profile superior to conventional therapy in the MIA-induced osteoarthritis model. The findings support its potential as a promising, safe, polyherbal candidate for disease modification in osteoarthritis and justify further evaluation in chronic models and clinical trials.

Keywords: Analgesic activity, Anti-arthritic, Inflammation, MIA model, Osteoarthritis, OstoCan-V50, Polyherbal formulation, Wistar rats.

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Introduction

Osteoarthritis (OA), a chronic degenerative joint disorder, is a leading cause of disability worldwide, characterized by progressive cartilage degradation, synovial inflammation, subchondral bone remodelling, and osteophyte formation (1). These pathological changes manifest as joint pain, stiffness, and reduced mobility, profoundly impacting patients' quality of life and imposing substantial socioeconomic burdens (2-4). The Global Burden of Disease Study estimates that OA affected approximately 606.5 million individuals globally from 1990 to 2021, with a disproportionate impact on women and individuals over 70 years, for whom it ranks as the seventh leading cause of disability (5, 6). The increasing prevalence of OA, driven by aging populations, rising obesity rates, sedentary lifestyles, and metabolic disorders, underscores the urgent need for effective therapeutic strategies to mitigate its impact (7).

The pathogenesis of osteoarthritis is multifaceted, involving biomechanical and biochemical mechanisms that disrupt joint homeostasis, with mechanical overload accelerating cartilage degradation. Inflammatory mediators such as Tumour Necrosis Factor-alpha (TNF-α), Interleukin-1 beta (IL-1β), and Interleukin-6 (IL-6) upregulate Cyclooxygenase-1 (COX-1) and

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Cyclooxygenase-2 (COX-2), thereby perpetuating chronic inflammation (8, 9). Associated with aging, genetics, trauma, obesity, and metabolic disorders, OA exhibits cartilage erosion, synovial hypertrophy, and subchondral bone sclerosis (10). Current treatments, including analgesics, Non-steroidal Anti-inflammatory Drugs (NSAIDs), corticosteroids, and physical therapy, focus on symptom relief but fail to modify disease progression, with risks of gastrointestinal, renal, and cardiovascular adverse effects (11-13). Non-pharmacological interventions provide limited benefits (14).

The shortcomings of conventional osteoarthritis treatments have incited interest in complementary and alternative medicine, particularly Ayurvedic polyherbal formulations, which combine multiple botanical constituents to achieve synergistic therapeutic effects, potentially offering safer and more effective alternatives to synthetic drugs (15). OstoCan-V50, a novel polyherbal tablet, comprises *Withania somnifera (L.)*, *Boswellia serrata Roxb. ex Coleb., Cissus quadrangularis (L.)*, *Kukkudandatwak Bhasma*, and *Cannabis sativa (L.)*. Each component has documented therapeutic properties in traditional and preliminary scientific literature. *Boswellia serrata Roxb. ex Coleb.* contains boswellic acids, which inhibit 5-lipoxygenase and reduce pro-inflammatory cytokine production (16,17). *Withania somnifera (L.)* modulates NF-κB pathways, *Cissus quadrangularis (L.)* supports cartilage and bone repair, *Kukkudandatwak Bhasma* enhances bone remodelling, and *Cannabis sativa (L.)* targets cannabinoid receptors, though its role in polyherbal formulations is underexplored (18-21).

The therapeutic efficacy and safety of polyherbal formulations for osteoarthritis, particularly those containing *Cannabis sativa (L.)*, remain poorly defined. Limited characterization of their synergistic effects and safety profiles underscores research gaps (8). The monosodium iodoacetate (MIA)-induced OA model in Wistar rats provides a robust preclinical platform to evaluate therapeutic interventions, as it recapitulates key features of human OA, including cartilage degradation, synovial inflammation, and pain-related behaviours (22). This model enables the assessment of both anabolic and catabolic processes, offering a comprehensive framework to investigate the therapeutic potential of novel agents (23). As the limitations of existing treatments and the promising but untested properties of OstoCan-V50, a preclinical evaluation of its efficacy and safety is both timely and necessary.

This study addresses critical gaps in the management of OA by evaluating the anti-arthritic potential of OstoCan-V50 in a preclinical model. Its significance lies in its potential to validate a polyherbal formulation as a disease-modifying therapy, offering a safer alternative to conventional treatments while contributing to the scientific foundation of Ayurvedic medicine. The objectives of this study are to assess the efficacy of OstoCan-V50 tablets in reducing joint inflammation, cartilage degradation, and pain in the MIA-induced osteoarthritis model in Wistar rats, and to evaluate the safety profile of OstoCan-V50 through histopathological and biochemical analyses. By achieving these objectives, this study aims to establish OstoCan-V50 as a viable therapeutic candidate for OA, paving the way for future clinical investigations.

Materials and Methods

This study was conducted in strict adherence to the ARRIVE guidelines 2.0 to ensure rigorous and transparent reporting of animal research (24). All laboratory animal handling and experimental procedures complied with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA). The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) under approval number DYPIPSR/IAEC/NOV/23-24/P-03. These measures ensured ethical treatment of animals, minimized distress, and maintained scientific integrity throughout the evaluation of OstoCan-V50's anti-arthritic activity in the MIA-induced osteoarthritis model in Wistar rats.

Test and Standard Drug

OstoCan-V50 tablets, a polyherbal formulation, were supplied by Sudhatatva Pharmacy, Dr. D.Y. Patil College of Ayurveda and Research, Pimpri, Pune, India. All raw herbal ingredients and *Kukkudandatwak Bhasma* used in the formulation were sourced from authenticated suppliers approved by the institution, and their identity and quality were confirmed through standard pharmacognostical and physicochemical evaluations. Each tablet of OstoCan-V50 contained powdered crude herbal materials of *Withania somnifera (L.)* Dunal (Ashwagandha root, 150 mg), *Boswellia serrata Roxb. ex Colebr.* (Sallaki resin, 150 mg), *Cissus quadrangularis (L.)* (Hadjod stem, 100 mg), *Cannabis sativa (L.)* (Vijaya leaf and seed, 50 mg), and *Kukkudandatwak Bhasma* (calcined eggshell prepared by classical Ayurvedic methods, 50 mg), along with excipients (quantity sufficient). Diclofenac sodium (batch no. DP-2298, expiry June 2025) was procured from Aagya Biotech Pvt. Ltd., Pune, India. Enzyme-linked immunosorbent assay (ELISA) kits for TNF-α (catalog no. KB3145) and IL-1β (catalog no. KLR0119) were obtained from KRISHGEN BioSystems, Mumbai, India. Monosodium iodoacetate (MIA) and all other chemicals used were of analytical grade and high purity.

Quality Control Analysis of OstoCan V50 Tablet

The physical characteristics and quality control parameters of OstoCan-V50 tablets were systematically evaluated. Physicochemical parameters of all raw materials were evaluated following Ayurvedic Pharmacopoeia standards. Loss on drying was within the acceptable limit of ≤10%, total ash ≤12%, acid-insoluble ash ≤4%, and pH within the range of 5.5-7.5. Microbial load was below permissible limits (bacteria $<10^3$ CFU/g; fungi $<10^2$ CFU/g). Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, glycosides, and saponins. The tablets were packaged in plastic containers and presented a brown colour with a characteristic odor and a slightly astringent taste. They were oval-shaped, with an average thickness of 0.5 cm and a diameter of 1.6 cm. Quality control assessments demonstrated compliance with specified standards, including average weight, pH, hardness, and disintegration time, ensuring the tablets' consistency and efficacy.

Animals models

Female Wistar rats were housed in groups of 6-8 under controlled laboratory conditions, including a temperature of 25 ± 2°C and a 12-hour light/dark cycle, with ad libitum access to a standard pellet diet and drinking water as specified in the study protocol. All animal handling and experimental procedures were conducted in strict compliance with the guidelines.

Acute Oral Toxicity Study

An acute oral toxicity study was conducted following OECD Guideline 423 to evaluate the safety of OstoCan-V50. Nine female Wistar rats were allocated to two groups: a control group (n=3) receiving the vehicle (1 mL, oral) and a test group (n=6) administered OstoCan-V50 at 2000 mg/kg body weight (oral).

Animals were observed individually post-dosing, initially within the first 30 minutes, for clinical signs, including changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, and central nervous systems, somatomotor activity, and behaviour. Observations continued periodically for the first 24 hours, with particular attention during the first 4 hours, and daily thereafter for 14 days. On day 15, gross necropsy was performed to examine vital organs (liver, kidney, heart, spleen, brain, adrenals, ovaries, lungs), which were preserved in 10% neutral buffered formalin. Organs exhibiting abnormalities were subjected to histopathological analysis.

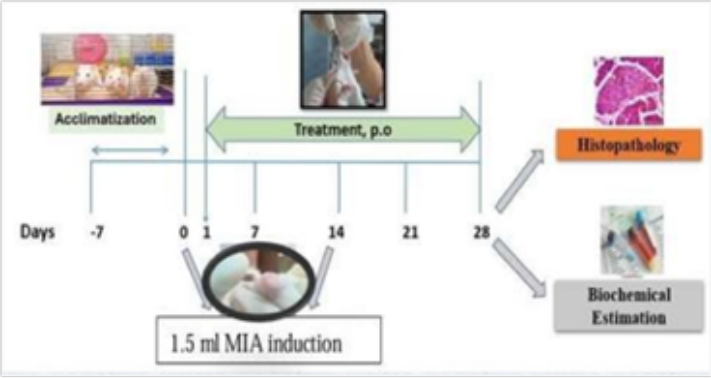
Experimental Design to study Anti-Arthritic Activity of OstoCan-V50

Wistar rats were randomly allocated to six groups (n=8 per group) to evaluate the anti-arthritic activity of OstoCan-V50 in a monosodium iodoacetate (MIA)-induced osteoarthritis model and treatment protocol summarized (**Figure 1**). On day 0, MIA was injected intra-articularly to induce osteoarthritis in groups 2-6. Treatments were administered orally daily for 28 days (**Table 1**). On day 28, animals were anesthetized with diethyl ether, blood was collected via retro-orbital puncture for biochemical analysis, and rats were subsequently euthanized to harvest hind limbs for histopathological examination.

Table 1: Representation of different treatment groups for study

Groups	Treatment	Dose & Route	Number of animals
G1	Normal control (0.25% Na-CMC)	10 ml/kg, p.o.	8
G2	Disease control MIA (1.5 mg/50ul intra articular) + (0.25% Na- CMC)	10 ml/kg, p.o.	8
G3	MIA (1.5 mg//50ul intra articular) + Diclofenac	10 ml/kg, p.o.	8
G4	MIA (1.5 mg//50ul intra articular) + Test compound	25.75 mg/kg, p.o.	8
G5	MIA(1.5 mg//50ul intra articular) + Test compound	12.87 mg/kg, p.o.	8
G6	MIA(1.5 mg//50ul intra articular) + Test compound	51.5 mg/kg, p.o.	8
	TOTAL		**48**

Figure 1: Study Design for evaluation of anti-arthritic activity



Evaluation Parameters

Clinical signs and mortality were monitored daily throughout the study. All animals were observed for clinical signs or mortality from day 0 to day 28 to detect any adverse effects of the treatments. Food and water intake were measured before arthritis induction and every alternate day from day 0 to day 28. These parameters were recorded to assess the impact of the MIA-induced osteoarthritis model and OstoCan-V50 treatment on nutritional status. Body weight was recorded before arthritis induction and every alternate day from day 0 to day 28. Regular monitoring of body weight provided insights into the systemic effects of the disease and treatment.

Pain threshold was evaluated using the hot plate method on days 0, 7, 14, and 28. This test assessed the analgesic efficacy of OstoCan-V50 by measuring the latency of response to thermal stimuli. Knee joint thickness was measured daily after MIA injection using a Vernier caliper. The width of injected and non-injected hind limbs was recorded above the knee joint, and the mean change relative to initial width was calculated on respective days compared with the untreated group.

Complete blood count (CBC) was performed on day 28. Blood samples, collected via retro-orbital puncture, were analysed to assess haematological parameters in the osteoarthritis model. Serum cytokine levels were quantified on day 28. Serum, separated from blood samples collected via retro-orbital puncture, was processed for TNF-α and IL-1β by ELISA kits.

Radiological evaluation was conducted on day 28. Digital X-ray images of knee joints were analysed for soft tissue swelling, periosteal reaction, joint space narrowing, periarticular osteoporosis, bone erosions, and other lesions, with abnormalities graded as normal (0), slight (1), moderate (2), or severe (3). Histopathological examination was performed on day 28. After euthanasia, hind limbs were harvested, fixed in 10% buffered formalin, and processed for paraffin embedding. Sections of 3-5µm thickness were cut, stained with haematoxylin and eosin, and examined by a board-certified toxicopathologist.

Statistical analysis

Data were analysed using GraphPad Prism (version 8.0) with one-way analysis of variance (ANOVA) followed by post hoc multiple comparison tests (25, 26). Results were expressed as mean ± standard error of the mean (SEM). Comparisons were performed in three ways: (1) the normal group was compared with the disease (MIA-induced osteoarthritis model), standard (diclofenac), and all test (OstoCan-V50) groups; (2) the disease group was compared with the normal, standard, and all test groups; and (3) the standard group was compared with all test groups. A p-value < 0.05 was considered statistically significant.

Results

High dose OstoCan-V50 treatment is safe in Wistar rats

OstoCan-V50 tablets were administered orally at a dose of 2000 mg/kg to female Wistar rats. No mortality was observed, and no notable changes in skin, fur, eyes, salivation, or mucous membranes were recorded. Sedation and grooming behaviours were noted, suggesting pharmacological activity of the formulation. Daily food and water intake were normal. These findings indicate that a single oral dose of OstoCan-V50 was non-toxic and safe in Wistar rats.

OstoCan-V50 increases food intake in MIA-induced OA

Food intake was measured every alternate day from day 0 to 28 day. MIA-induced osteoarthritis group exhibited significantly reduced food intake compared with the normal control group ($P < 0.01$). In contrast, the standard treatment group (diclofenac, 10 mg/kg) and OstoCan-V50 treatment groups (12.87 mg/kg, 25.75 mg/kg, 51.5 mg/kg) demonstrated significantly increased food intake compared with the disease control group ($P < 0.05$) (**Figure 2**). OstoCan-V50 showed a dose-dependent increase in food intake, with the greatest effect observed at the 51.5 mg/kg dose. Statistical comparisons were conducted in three ways: (1) the normal control group was compared with the disease, standard, and all OstoCan-V50 test groups with $P < 0.05$, $P < 0.01$, $**P < 0.001$, $***P < 0.0001$, respectively; (2) the disease group was compared with the normal, standard, and all test groups, with results reported as nonsignificant; and (3) the standard group was compared with all test groups, with results reported as nonsignificant.

OstoCan-V50 increases water intake in MIA-induced OA

Water intake was measured every alternate day from day 0 to 28 day. Treatment with OstoCan-V50 and diclofenac significantly increased water intake compared with the disease control group. A significant difference in water intake was observed on days 21 and 28 at the 51.5 mg/kg dose of OstoCan-V50 ($P < 0.01$) (**Figure 3**). Statistical comparisons were conducted in three ways: (1) the normal control group was compared with the disease, standard (diclofenac), and all OstoCan-V50 test groups ($P < 0.01$); (2) the disease group was compared with the normal, standard, and all test groups, with significance denoted as $P < 0.05$, $P < 0.01$, $**P < 0.001$, respectively; and (3) the standard group was compared with all test groups, with results reported as nonsignificant.

OstoCan-V50 increases body weight in MIA-induced OA

Body weight was measured every alternate day from day 0 to 28 day. MIA-induced osteoarthritis disease control group exhibited significantly reduced body weight compared with the normal control group ($P < 0.01$). The OstoCan-V50 (51.5 mg/kg) and diclofenac (10 mg/kg) treatment groups demonstrated significant body weight gain compared with the disease control group ($P < 0.05$), with the most pronounced effect observed on days 21 and 28 (**Figure 4**). OstoCan-V50 demonstrated efficacy comparable to the standard treatment, significantly improving disease-related outcomes.

OstoCan-V50 treatment alleviates knee joint inflammation MIA-induced OA

Knee joint thickness, an indicator of inflammation, was measured using a Vernier caliper on days 0, 7, 21, and 28 in the MIA-induced osteoarthritis model. On day 0, all groups showed comparable thickness, but by day 7, the disease control group exhibited significantly increased thickness (11.6 ± 0.1 mm, $***P < 0.0001$) compared with the standard treatment group (diclofenac, 10 mg/kg; 11.5 ± 0.0 mm), and by day 28, the disease control group's thickness further increased (11.7 ± 0.1 mm, $***P < 0.0001$), whereas OstoCan-V50 treatment groups (12.87 mg/kg: 10.9 ± 0.1 mm; 25.75 mg/kg: 11.5 ± 0.1 mm) and the standard group showed significant reductions ($P < 0.0001$) (**Figure 5**) compared with the disease control group, with OstoCan-V50 demonstrating a dose-dependent improvement in joint inflammation, suggesting its efficacy in attenuating osteoarthritis-related inflammation.

OstoCan-V50 treatment increases pain threshold in MIA-induced OA

Pain threshold was assessed using the thermal stimulus response (hot plate method) over 60 minutes in the MIA-induced osteoarthritis model (**Figure S1**); the MIA-treated disease control group showed no significant change in response time, whereas the standard (diclofenac, 10 mg/kg) and OstoCan-V50 treatment groups (12.87 mg/kg, 25.75 mg/kg, 51.5 mg/kg) exhibited significantly increased response times ($***P < 0.0001$), indicating a sustained analgesic effect lasting at least 1 hour post-administration, with a dose-dependent improvement in pain threshold and the greatest response observed at 51.5 mg/kg.

OstoCan-V50 treatment reduces inflammatory cytokines in MIA-induced OA

Serum levels of TNF-α and IL-1β were quantified on day 28 using ELISA. MIA-induced osteoarthritis disease control group exhibited significantly elevated TNF-α and IL-1β levels compared with the normal control group ($P < 0.0001$), whereas the standard treatment (diclofenac, 10 mg/kg) and OstoCan-V50 treatment groups (12.87 mg/kg, 25.75 mg/kg, 51.5 mg/kg) showed significantly reduced TNF-α and IL-1β levels compared with the disease control group ($P < 0.0001$), with the 51.5 mg/kg dose of OstoCan-V50 demonstrating a greater reduction compared with lower doses (**Figure S2**). All treatment groups showed significant differences compared to controls, with the standard and test groups demonstrating marked efficacy in improving disease parameters.

OstoCan-V50 improves haematological parameters in MIA-induced OA

CBC parameters were assessed on day 28 in the MIA-induced osteoarthritis model. The disease control group treated with MIA exhibited significant decreases in RBC and haemoglobin levels, alongside significant increases in WBC and lymphocyte counts compared with the normal control group ($P < 0.0001$). In contrast, the standard treatment (diclofenac, 10 mg/kg) and OstoCan-V50 treatment groups (12.87 mg/kg, 25.75 mg/kg, and 51.5 mg/kg)

Figure 2: **OstoCan-V50 showed a dose-dependent increase in food intake**

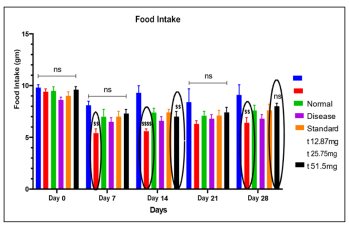


Figure 3: Effect of OstoCan-V50 tablets on Water intake in MIA induced OA in Wistar rats

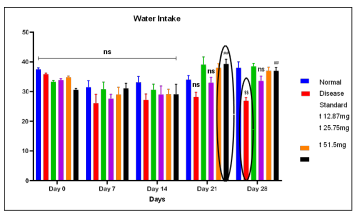


Figure 4: Effect of OstoCan-V50 tablets on Body weight in MIA induced OA in Wistar rats

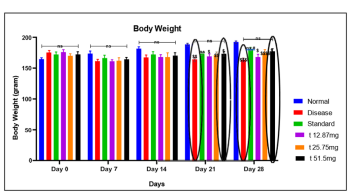
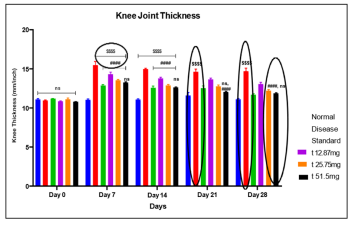


Figure 5: Effect of OstoCan-V50 tablets on Knee joint thickness in MIA induced OA in Wistar rats



demonstrated significant increases in RBC and haemoglobin levels, as well as reductions in WBC and lymphocyte counts compared with the disease control group ($P < 0.0001$) (**Figure S3**).

OstoCan-V50 mitigates joint damage in MIA-induced OA

The radiological examination of the normal control group revealed no pathological lesions (**Table 2**). The MIA-treated disease control group exhibited soft tissue swelling, periosteal hypertrophy, joint space narrowing, periarticular osteoporosis, and bone erosions. Both the standard treatment and OstoCan-V50 groups showed similar findings but with reduced severity, suggesting that OstoCan-V50 mitigates joint damage and may support cartilage regeneration (**Figure S4**).

Table 2: Radiological observations of knee joints

Group	Swelling of soft tissue around joints	Peri-osteal reaction	Joint space narrowing	Bone erosion	Osteoporosis
Normal Control	0	0	0	0	0
Disease Control	3	3	2	2	1
Standard	1	1	1	1	1
t (12.85mg)	2	0	1	1	2
t(25.75mg)	2	0	2	2	2
t(51.5mg)	1	1	1	1	1

Figure S1: Effect of OstoCan-V50 tablets on Pain threshold in MIA induced OA in Wistar rats

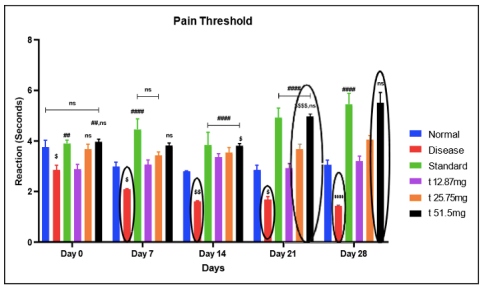


Figure S2: Effect of OstoCan-V50 Tablets on (a) TNF-α and (b) IL-1β Levels in MIA-Induced Osteoarthritis in Wistar Rats

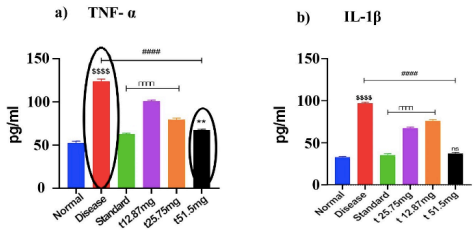


Figure S3: Effect of OstoCan-V50 tablets on the level of (A)RBCs, (B)WBCs in MIA induced OA in Wistar rats

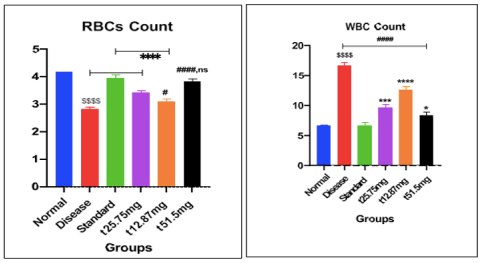


Figure S4: **X-ray imaging of Knee joint of Wistar rats. Digital X-ray imaging analysis to evaluate joint damage in MIA-induced OA model after 28 days. (a) Normal control, (b) Disease control, (c) Standard treatment (diclofenac), (d) Treatment 1(12.85 mg), (e) Treatment 2 (25.75 mg), and (f) Treatment 3 (51.5 mg)**

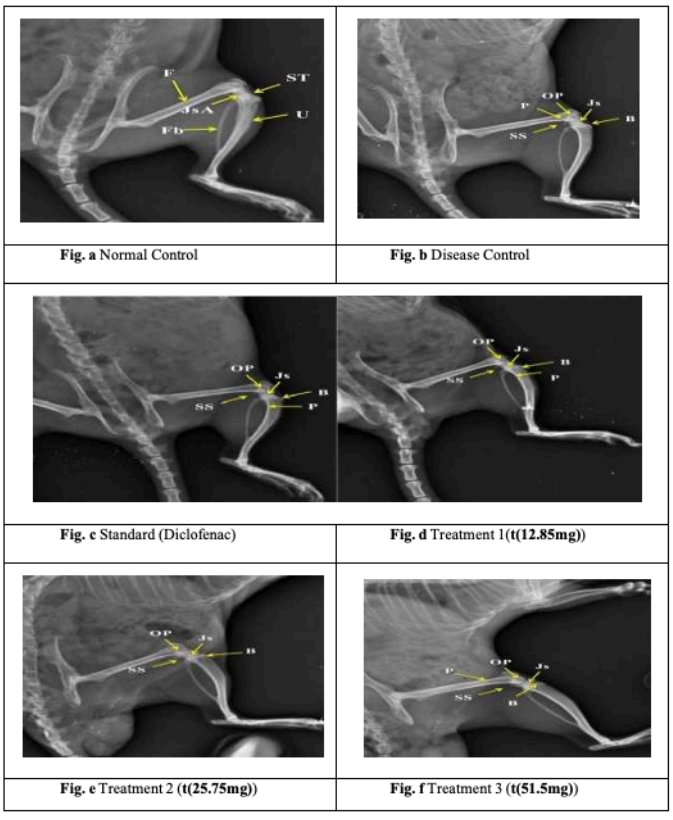


Fig. a Normal Control

Fig. b Disease Control

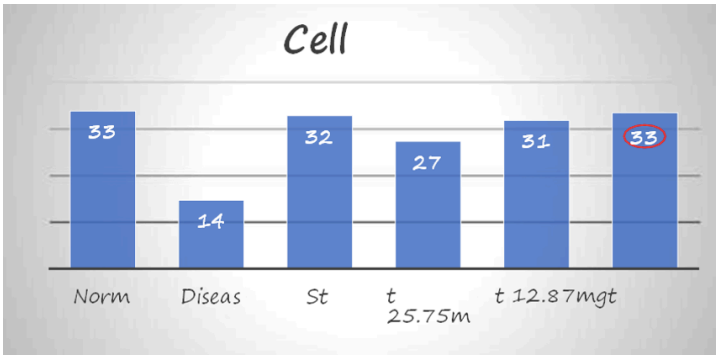
Fig. c Standard (Diclofenac)

Fig. d Treatment 1(**t(12.85mg)**)

Fig. e Treatment 2 (**t(25.75mg)**)

Fig. f Treatment 3 (**t(51.5mg)**)

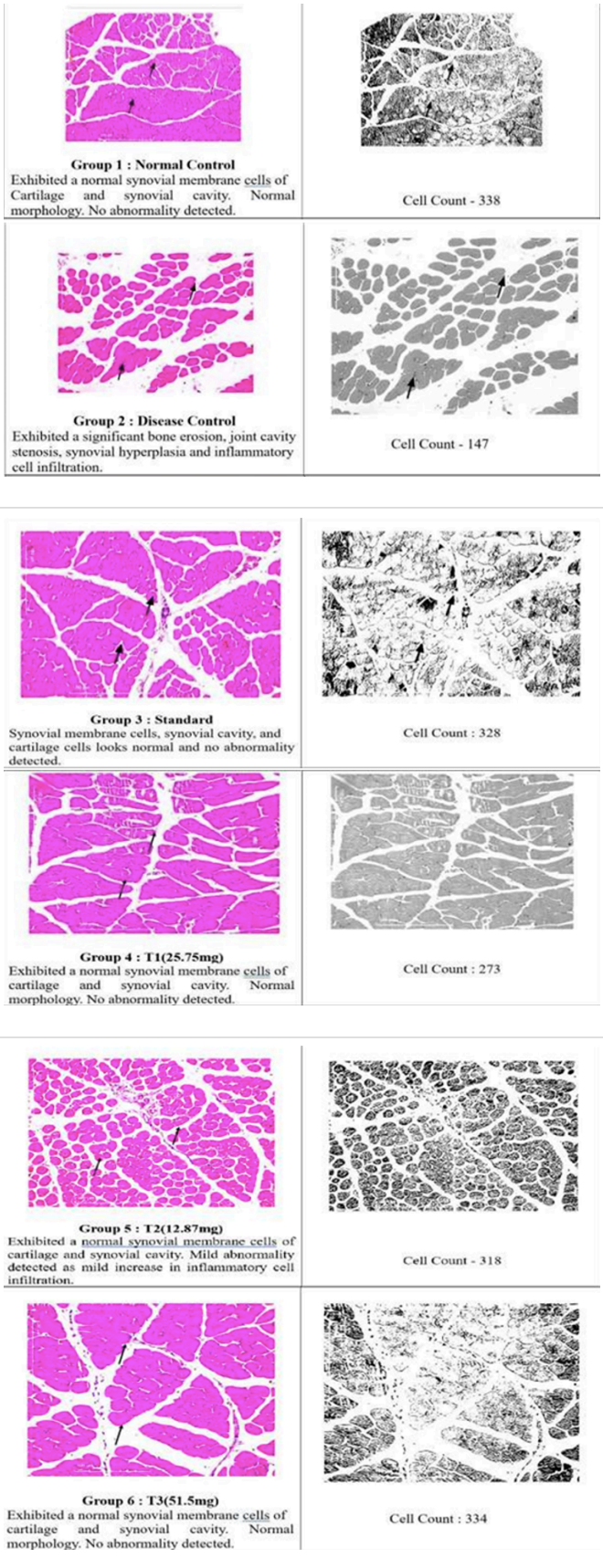
Figure S5: Cell Count of Knee Joint



OstoCan-V50 reduces joint damage and improves cell viability in OA.

Histopathological analysis on day 28 in the MIA-induced osteoarthritis model revealed severe inflammation, synovial infiltration, joint swelling, pain, and deformities in the disease control group; in contrast, OstoCan-V50-treated groups (12.87 mg/kg, 25.75 mg/kg, 51.5 mg/kg) exhibited near-normal joint architecture with minimal surface damage, while ImageJ analysis (version 1.54j) quantified viable cell counts based on pixel thresholds, showing a significant reduction in the disease control group (147 cells, 62% lower than the normal control group's 338 cells) (Figure S5), with the standard treatment group (diclofenac, 10 mg/kg) displaying the highest viable cell count (338 cells), followed by OstoCan-V50 at 51.5 mg/kg (334 cells), 12.87 mg/kg (318 cells), and 25.75 mg/kg (273 cells), indicating that OstoCan-V50, particularly at higher doses, effectively preserved cell viability compared with the disease control group (Figure S6).

Figure S6: Histopathological images and Image J of different treatment groups. Histopathological images and Image J of different treatment groups. Images of HE stained tissue sections of control and treatment groups (Left), the respective Image J analysis for histopathological sections with cell counts (Right).



Discussion

This study provides for the anti-arthritic and analgesic efficacy of OstoCan-V50, a novel polyherbal tablet, in the MIA-induced osteoarthritis model in Wistar rats. Administered at doses of 12.87, 25.75, and 51.5 mg/kg, OstoCan-V50 significantly reduced knee joint inflammation, mitigated cartilage degradation, and alleviated pain, with the 51.5 mg/kg dose showing effects comparable to diclofenac (10 mg/kg). Biochemical analyses demonstrated marked reductions in pro-inflammatory cytokines (TNF-α and IL-1β) in treated groups compared with disease controls ($P < .0001$). Radiological and histopathological assessments further confirmed reduced joint damage, preserved cartilage architecture, and enhanced cell viability, particularly at the highest dose. The acute oral toxicity study established the safety of OstoCan-V50 at 2000 mg/kg, with no observed mortality or significant adverse effects. These findings collectively emphasise OstoCan-V50's potential as a safe and effective therapeutic agent for osteoarthritis management.

Osteoarthritis is driven by complex inflammatory and degenerative processes, including cartilage erosion, synovial inflammation, and subchondral bone remodelling. The MIA model effectively recapitulates these features, enabling evaluation of both symptomatic and structural outcomes (8, 27-29). The significant reduction in knee joint thickness observed with OstoCan-V50 treatment aligns with its ability to modulate inflammatory pathways, as evidenced by decreased TNF-α and IL-1β levels. These cytokines play pivotal roles in upregulating matrix metalloproteinases and cyclooxygenases, which perpetuate cartilage degradation and pain (30). The dose-dependent efficacy of OstoCan-V50, particularly at 51.5 mg/kg, suggests its capacity to target these mediators, offering a dual benefit in alleviating inflammation and preserving joint integrity. The formulation's analgesic effects, demonstrated by increased pain threshold latency in the hot plate test, further support its potential to address the pain component of osteoarthritis, a critical factor affecting patients' quality of life.

OstoCan-V50's efficacy is due to its polyherbal constituents *Withania somnifera (L.)*, Boswellia serrata Roxb. ex Coleb., *Cissus quadrangularis (L.)*, *Kukkudandatwak Bhasma, and Cannabis sativa (L.)*. Boswellic acids inhibit 5-lipoxygenase, reducing inflammation, while *Withania somnifera* modulates NF-κB signalling. *Cissus quadrangularis (L.)* supports cartilage repair, and *Kukkudandatwak Bhasma* promotes bone remodelling. *Cannabis sativa (L.)* may provide analgesia via cannabinoid receptors, though its role requires further study (16, 19, 31-33). These combined actions target multiple pathways, potentially offering benefits over NSAIDs, which carry gastrointestinal and cardiovascular risks (34). The comparable efficacy of OstoCan-V50 to diclofenac, combined with its favourable safety profile, positions it as a promising alternative for osteoarthritis management, particularly for patient's intolerant to conventional treatments.

OstoCan-V50 demonstrates a comprehensive therapeutic impact extending beyond symptomatic relief to systemic and structural restoration in osteoarthritis. In the MIA model, characteristic reductions in food and water intake and body weight markers of cachexia and metabolic distress were significantly reversed by OstoCan-V50 at 51.5 mg/kg. Haematological parameters showed decreased white blood cell and lymphocyte counts, indicating reduced inflammatory activation. Radiological assessments revealed mitigation of joint space narrowing and bone erosion, while histopathological analysis confirmed cartilage preservation

and increased viable cell populations. These results suggest that OstoCan-V50 may offer disease-modifying benefits by targeting both clinical symptoms and underlying pathology.

Despite these encouraging findings, the MIA model, although suitable for acute and subacute osteoarthritis, lacks the ability to fully replicate the chronic nature of human disease, indicating the need for additional evaluation in chronic models. The acute toxicity study at 2000 mg/kg supports short-term safety, but long-term toxicity studies are essential to confirm safety for prolonged use, given the polyherbal nature of OstoCan-V50. The bioavailability and pharmacokinetics of its active constituents remain unexplored, which are critical for optimizing dosing regimens and ensuring clinical efficacy. Additionally, while the study demonstrates significant anti-inflammatory and analgesic effects, the specific contributions of individual herbal components and their synergistic interactions require further investigation. Future studies should employ multi-omics approaches, such as transcriptomics to assess NF-κB pathway modulation, proteomics to quantify inflammatory mediators, and metabolomics to evaluate changes in lipid mediators like prostaglandins. These approaches would elucidate the molecular mechanisms underlying OstoCan-V50's efficacy, bridging traditional Ayurvedic knowledge with modern systems biology.

Innovative therapeutic strategies are essential to address the multifactorial nature of osteoarthritis, and polyherbal formulations such as OstoCan-V50 offer promising potential results. This study substantiates the efficacy and safety of OstoCan-V50, contributing to efforts against osteoarthritis. The formulation may serve as a safer alternative to nonsteroidal anti-inflammatory drugs (NSAIDs), responding to the critical need for therapies with improved safety profiles. Rigorous quality control and standardized preclinical evaluation, as conducted herein, strengthen the scientific validation of Ayurvedic medicines and facilitate their integration into clinical practice. Further investigation through clinical trials in osteoarthritis patients, particularly those with comorbidities, is warranted to confirm these findings. Additionally, evaluating OstoCan-V50 as an adjunctive treatment could enhance therapeutic outcomes by addressing the complex pathophysiology of osteoarthritis.

Conclusion

In this preclinical study, OstoCan-V50, a novel polyherbal formulation, demonstrated significant anti-arthritic and analgesic efficacy in the monosodium iodoacetate-induced osteoarthritis model in Wistar rats. Administered at doses of 12.87, 25.75, and 51.5 mg/kg, OstoCan-V50 significantly reduced knee joint inflammation, cartilage degradation, and pain, with the 51.5 mg/kg dose showing effects comparable to diclofenac (10 mg/kg). Biochemical analyses revealed marked reductions in proinflammatory cytokines, while radiological and histopathological assessments confirmed attenuated joint damage and preserved cartilage architecture. Haematological improvements and increased food intake, water intake, and body weight further underscored its systemic benefits. An acute oral toxicity study established the safety of OstoCan-V50 at 2000 mg/kg, with no mortality or significant adverse effects. These findings suggest that OstoCan-V50 holds promise as a safe and effective therapeutic candidate for osteoarthritis management, warranting further investigation through chronic models and clinical trials to validate its disease-modifying potential and long-term safety.

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