RESEARCH PAPER

Evaluation of Anti-inflammatory activity & Anti-arthritic Activity of hydro-alcoholic Bark Extract of Moringa concanensis in complete Freund's Adjuvant induced arthritic rats

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ABSTRACT

Rheumatoid arthritis is a long-term autoimmune disorder marked by persistent inflammation of synovial joints, resulting in progressive joint damage and functional disability. Current pharmacological treatments provide relief but are often associated with adverse effects during prolonged use. This study investigated the anti-arthritic potential of a hydro alcoholic bark extract of Moringa concanensis using complete Freund's adjuvant (CFA) induced arthritis in Wistar rats. Arthritis was induced by sub plantar injection of CFA and treatment was administered orally for 21 days. Parameters including paw edema, arthritic score, body weight, hematological indices, biochemical markers and histopathology of joint tissues were assessed. The extract produced a significant reduction ($p<0.05$) in paw swelling and arthritic index, improved body weight and restored altered hematological and biochemical parameters. Histological analysis indicated reduced inflammatory cell infiltration and protection against cartilage degradation. These findings suggest that Moringa concanensis bark extract possess notable anti-arthritic activity, possibly mediated through anti-inflammatory and antioxidant mechanisms.

Results: Wistar Rats receiving the hydro-alcoholic extract at dosages of 100mg/kg (p.o), 200mg/kg (p.o) and 400mg/kg (p.o) exhibited a notable reduction in the physical and biochemical indicators of arthritis in comparison to the untreated arthritic model. Histopathological observations further confirmed that the anti-arthritic effects of the hydro-alcoholic extract were on par with methotrexate.

Conclusion: The findings of this study indicate that hydro-alcoholic bark extract of Moringa concanensis exhibits significant anti-arthritic activity in experimental rat animals. The results support its traditional use and suggest its potential as a safer alternative for the management of rheumatoid arthritis.

KEYWORDS: Moringa concanensis, Rheumatoid arthritis, CFA model, Anti-inflammatory, Hydroalcoholic extract.

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INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory autoimmune disease primarily affecting joints, leading to pain, swelling, stiffness, and eventual joint destruction[2]. It involves complex immunological processes, including cytokine release, oxidative stress, and synovial hyperplasia. Despite advances in treatment, commonly used drugs such as NSAIDs, corticosteroids, and DMARDs may cause adverse reactions, especially with long term therapy[4].

Natural products derived from medicinal plants have gained attention due to their relatively safer profiles and therapeutic potential. *Moringa concanensis* is traditionally used for treating inflammatory conditions. It is reported to contain bioactive constituents such as flavonoids, phenolics, and alkaloids, which may contribute to its pharmacological effects. However, systematic scientific evaluation of its anti-arthritic activity remains limited[1,3].

Medicinal plants have been extensively investigated as alternative therapeutic agents due to their safety and efficacy. *Moringa concanensis*, belonging to the family Moringaceae, is traditionally used for its anti-inflammatory, analgesic, and antioxidant properties. The present study was undertaken to evaluate the anti-arthritic efficacy of hydro-alcoholic bark extract of *Moringa concanensis* in a CFA-induced arthritis model in Wistar rats[4].

MATERIAL AND METHODS

Plant Material and Extraction

The bark of *Moringa concanensis* was collected in desert area of Jodhpur, Rajasthan and authenticated then dried in shade. The powdered material was extracted with the hydroalcoholic solvent (70% Ethanol: 30% water) by Soxhlet apparatus and produced the highest yield (14.6% w/w) that is indicating the synergistic effect of ethanol and water in dissolving a wider range of polar and semi-polar phytoconstituents. The extract was concentrated under reduced pressure and stored in a desiccator until use[5].

Experimental Animals

The Anti-inflammatory activity studies were conducted using Wistar rats, a well-accepted experimental model due to their physiological and metabolic similarity to humans in inflammatory pathways. Animals used in the study were of either sex, aged between 6–8 weeks, weighing 180–220 g, and were housed under standard laboratory conditions (12 h light/dark cycle, temperature 24 ± 2 °C, relative humidity 50–60%). The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA guidelines[6].

Acute Toxicity Studies:

Acute toxicity tests were conducted on 6 female Wistar rats (180-220g) following OECD Guidelines 423, using a 200mg/kg dosage of an extract. Post-administration, the rats were closely monitored for 14days, with an initial intensive observation period of 4 hours for immediate behavioral changes such as convulsions, diarrhea, respiratory issues followed by 72 hours of mortality surveillance[7].

FCA-Induced Arthritis in Wistar Rats:

For this experimental assessment, Wistar rats were randomly assigned to five distinct groups, each encompassing six animals:

Group I: Normal control, with no arthritis induction.

Group II: Arthritis was induced using FCA, and no treatment was administered, thus serving as the positive control.

Group III: Rats with FCA-induced arthritis were treated with the standard methotrexate at a dosage of 0.75 mg/kg body weight.

Group IV: Rats with FCA-induced arthritis were treated with *Moringa concanensis* root extract at a concentration of 200mg/kg body weight.

Group V: Rats with FCA-induced arthritis received *Moringa concanensis* root extract at a dose of 400 mg/kg body weight.

Arthritis was experimentally induced by subcutaneous injection of 0.1ml of FCA into the sub-plantar region of the left hind paw on the first day of the study[15]. Following the induction, treatments as per group specifications were initiated on the subsequent day and continued for a span of 14 days. Post-treatment, a further observational period was extended till the 21stday to gauge the longer-term effects and potential mitigations. The anti-arthritic efficacy of the *Moringa concanensis* root extract was determined by evaluating several biophysical parameters, including the arthritic score, fluctuations in body weight, changes in paw volume, and variations in joint diameter[8].

Paw Volume Measurement:

The volume of the animals' left hind paws was measured using a specialized plethysmometer. Measurements were taken before the FCA injection on the initial day and again on the 21st day at set intervals. The overall change in paw volume was determined by subtracting the initial measurement from the final one[9].

Histopathological Examination of Liver and Ankle Joint:

On the 21st day, marking the conclusion of the study, the animals were euthanized in a humane manner. Afterward, their liver and ankle joints were meticulously removed and promptly stored in a 10% buffered formalin solution, making them ready for later histological examinations[10,11].

Statistical Analysis:

The documented results are expressed as mean ± SEM. Statistical comparisons were made between the treated groups and the arthritic control group. Data were analyzed through a one-way analysis of variance, followed by post hoc testing using GraphPad Prism

8.0.2 software. A p-value below 0.05 ($P<0.05$) was considered statistically significant.

Induction of Arthritis

Arthritis was induced by injecting 0.1 mL of complete Freund's adjuvant into the sub plantar region of the left hind paw. Development of arthritis was confirmed by swelling and redness[12].

RERULT:

phytoconstituents present in the *Moringa concanensis bark* extract:
The presented phytoconstituents finding from the phytochemical analysis of the *Moringa concanensis* bark extract.

Table 2.1: Qualitative Phytochemical Screening of *Moringa concanensis* Bark Extracts

Phytochemical Test	Methanolic Extract	Ethanolic Extract	Hydroalcoholic Extract
Alkaloids (Dragendorff's/ Wagner's)	+++	++ (Moderate)	+++ (Strong)
Flavonoids (Shinoda/$AlCl_3$ test)	+++	++	+++
Tannins (Ferric chloride test)	++	++	+++
Saponins (Froth test)	++	+	++
Phenolics (Ferric chloride test)	+++	++	+++
Glycosides (Keller–Killiani test)	++	++	+++
Terpenoids (Salkowski's test)	++	++	+++

(+++) Indicates the strong presence of chemical constituents

Experimental Design

Animals were randomly divided into Six groups (n=7):

Table 2.1: Experimental Group Design

Group	Treatment
Normal Control	No induction, no treatment
Disease Control	Carrageenan / CFA induced, untreated
Standard Drug (Diclofenac sodium)	Diclofenac sodium (10 mg/kg, oral)
Test Group I (Low Dose Extract)	Extract low dose (100 mg/kg, oral)
Test Group II (Medium Dose Extract)	Extract medium dose (200 mg/kg, oral)
Test Group III (High Dose Extract)	Extract high dose (400 mg/kg, oral)
Test Group IV (Gel Formulation)	Topical gel formulation (2% w/w applied locally)

All treatments were administered orally for 21 consecutive days.

Evaluation Parameters

Parameters Measured

The following parameters were recorded systematically:

- Paw volume and diameter to quantify the extent of edema and swelling.
- Arthritic index score, based on erythema, swelling, and joint mobility, providing a semi-quantitative measure of disease progression.
- Biochemical markers, including C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and erythrocyte sedimentation rate (ESR), were estimated as indicators of systemic inflammation.
- Body weight, food intake, and behavioral changes were monitored to evaluate the general health status and systemic well-being of the animals.

Table 2.1 outlines the experimental group design adopted for the in vivo anti-inflammatory and anti-arthritic studies. A total of seven groups were included

to provide comprehensive comparisons. The normal control group received neither disease induction nor treatment, serving as the baseline reference for physiological parameters. The disease control group was induced with carrageenan or CFA but left untreated, thus demonstrating the full extent of inflammatory or arthritic changes. The standard drug group received diclofenac sodium, a non-steroidal anti-inflammatory drug (NSAID), which served as the reference standard for anti-inflammatory efficacy. Four test groups were included three received graded oral doses of the purified extract (100, 200, and 400 mg/kg), while the fourth received the optimized topical gel formulation containing 2% w/w of the extract. This systematic grouping allowed for comparison across untreated, standard-treated, and experimental-treated conditions, thereby validating the therapeutic potential of both oral and topical formulations.

Category	Parameters	Methods Used
Inflammatory Signs	Paw volume, Paw diameter	Plethysmometer, Vernier calipers
Arthritic Progression	Arthritic index score	Semi-quantitative scoring system (0–4 scale)
Biochemical Markers	CRP, TNF-α, IL-6, ESR	ELISA kits, Automated analyzer
General Health	Body weight, Food intake, Behavioral changes	Daily observation and weekly measurement

Table 2.2: Parameters Measured

Table 2.2 Summarizes the parameters measured during in vivo pharmacological evaluation, categorized into inflammatory signs, arthritic progression, biochemical markers, and general health indicators. Inflammatory signs such as paw volume and diameter were measured using plethysmometer to quantify edema and swelling. Arthritic progression was evaluated through an arthritic index scoring system (0–4 scale), which provided a semi-quantitative measure of joint swelling, erythema, and mobility restriction. Biochemical markers including CRP, TNF-α, IL-6, and ESR were estimated using ELISA kits and automated analyzers to assess systemic inflammation at the molecular level. Finally, general health indicators such as body weight, food intake, and behavioral changes were monitored to evaluate the overall well-being of animals during treatment. Together, these parameters ensured a comprehensive assessment of both local and systemic therapeutic effects of the extract and gel formulation.

Figure 2.1: Effect of treatments on Paw Edema (Carrageenan Model)

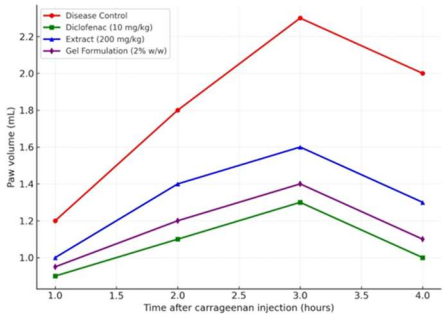

Figure 2.1 illustrates the effect of different treatments on carrageenan-induced paw edema in rats. The disease control group showed a sharp increase in paw volume, reaching a peak of approximately 2.3 mL at the third hour, indicating a strong inflammatory response. In contrast, the standard drug Diclofenac sodium significantly suppressed paw edema throughout the observation period, with volumes maintained between 0.9 and 1.3 mL, demonstrating its potent anti-inflammatory potential. The group treated with the plant extract exhibited moderate suppression of edema, with paw swelling reduced compared to the disease control but not as effectively as Diclofenac sodium. Interestingly, the gel formulation group displayed results nearly comparable to the standard drug, maintaining paw volumes much lower than the extract alone, thereby highlighting the improved bioavailability and therapeutic efficacy achieved through topical delivery. These findings suggest that both the extract and the gel possess anti-inflammatory properties, with the gel formulation offering superior performance close to that of the standard reference drug.

Figure2.2: Effect of treatments on Arthritic Index Progression (CFA Model)

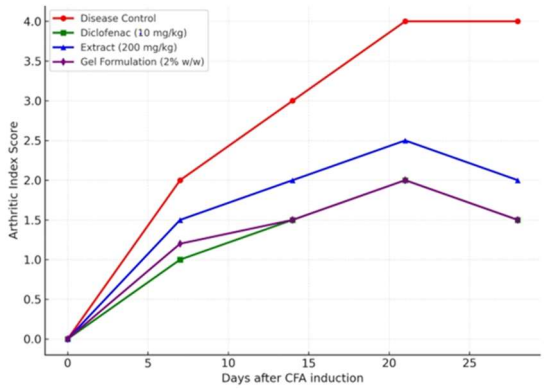

Figure 2.2 presents the progression of arthritic index scores in CFA-induced arthritis over a period of 28 days. The disease control group displayed a steady increase in severity, reaching the maximum arthritic index score of 4 by day 21 and persisting through day 28, indicative of severe chronic inflammation and joint damage. Treatment with Diclofenac markedly suppressed the progression of arthritis, with scores not exceeding 2 throughout the experimental period, confirming its well-established anti-arthritic action. The plant extract group showed a moderate reduction in arthritic symptoms, peaking at a score of 2.5, reflecting partial protection against joint inflammation. The gel formulation, however, produced superior results compared to the extract alone, limiting the maximum arthritic index to around 2, which closely matched the response seen with Diclofenac. These observations clearly establish that the gel formulation not only enhances the therapeutic potential of the extract but also provides a level of efficacy approaching that of the standard anti-inflammatory drug.

The combined use of acute and chronic models ensured a comprehensive assessment of anti-inflammatory and anti-arthritic efficacy. While the carrageenan model focused on acute mediators of inflammation, the CFA model provided insights into chronic immune-mediated pathology. This multi-model in vivo approach validated the therapeutic potential of the extract and its gel formulation, supporting their application as a novel anti-inflammatory intervention.Paw volume was measured using a plethysmometer on days 0, 7, 14, and 21.

Arthritic Index: Carrageenan-Induced Paw Edema Model (Acute Inflammation)

The carrageenan-induced paw edema model was employed to evaluate the acute anti-inflammatory effect of *Moringa concanensis* bark extracts. Paw volume was measured at regular intervals after carrageenan injection.

Table 2.3: Effect of M. concanensis Extracts on Carrageenan-Induced Paw Edema in Rats

Treatment Group	Paw Volume (mL) at 3 h	%Inhibition of Edema
Control (Normal saline)	1.82 ± 0.04	–
Standard (Diclofenac sod. 10 mg/kg)	0.82 ± 0.03	54.9
Hydroalcoholic Extract (200 mg/kg)	0.91 ± 0.04	50.0
Methanolic Extract (200 mg/kg)	1.04 ± 0.05	42.8
Ethanolic Extract (200 mg/kg)	1.21 ± 0.04	33.5

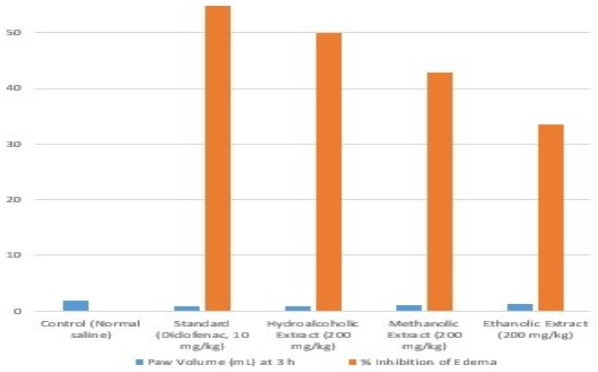

Figure 2.3: Effect of Extracts on Paw Edema (3h post-carrageenan injection)

The hydroalcoholic extract produced the maximum inhibition of paw edema (50%), comparable to the

standard drug diclofenac, confirming its strong acute anti-inflammatory potentia comparable to other solvent system.

Complete Freund's Adjuvant (CFA)-Induced Arthritis Model (Chronic Inflammation)

A. Arthritis Scoring

Arthritis severity was assessed using a standard scoring system (0–4 per paw, maximum score 16 per rat).

Table 2.4: Arthritis Scores in CFA-Induced Rats

Treatment Group	Mean Arthritis Score (Day 21)	% Reduction vs Arthritic Control
Arthritic Control (CFA only)	12.6 ± 0.4	–
Standard (Diclofenac, 10 mg/kg)	4.2 ± 0.3	66.7
Hydroalcoholic Extract (200 mg/kg)	4.8 ± 0.4	61.9
Methanolic Extract (200 mg/kg)	6.2 ± 0.5	50.8
Ethanolic Extract (200 mg/kg)	7.8 ± 0.4	38.1

Hydroalcoholic extract markedly reduced arthritis scores in a dose-dependent manner, nearly matching the standard drug.

B. Body Weight

Body weights were recorded at regular intervals during the study period. Body weight loss is a common marker of systemic inflammation and disease severity. Treatment with *M. concanensis* extracts improved body weight compared to arthritic control animals.

Table 4.19: Effect of Extracts on Body Weight in CFA-Induced Arthritic Rats

Group	Initial Weight (g)	Final Weight (g, Day 21)	% Change
Normal Control	198.2 ± 3.1	210.4 ± 3.5	+6.2
Arthritic Control (CFA)	197.5 ± 2.9	182.1 ± 3.2	–7.8
Standard (Diclofenac)	196.7 ± 3.2	205.6 ± 3.6	+4.5
Hydroalcoholic Extract	198.1 ± 3.0	203.7 ± 3.4	+2.8
Methanolic Extract	197.8 ± 3.1	200.6 ± 3.3	+1.4
Ethanolic Extract	198.0 ± 3.2	196.3 ± 3.5	–0.8

C. Hematological and Biochemical Parameters

Arthritic animals showed significant alterations in hematological parameters (increased WBC, decreased RBC and hemoglobin) and elevated inflammatory markers. Treatment with hydroalcoholic and methanolic extracts normalized these values toward normal ranges.

Table 4.20: Hematological and Biochemical Changes in CFA-Induced Arthritis

Group	Hb (g/dL)	RBC ($\times 10^6$/µL)	WBC ($\times 10^3$/µL)	ESR (mm/hr)	CRP (mg/L)
Normal Control	13.5 ± 0.4	7.1 ± 0.3	7.6 ± 0.5	9.2 ± 0.6	2.4 ± 0.3
Arthritic Control (CFA)	9.6 ± 0.3	4.8 ± 0.2	12.9 ± 0.7	22.6 ± 1.1	9.1 ± 0.4
Standard (Diclofenac sod.)	12.8 ± 0.4	6.7 ± 0.3	8.3 ± 0.6	11.3 ± 0.7	3.1 ± 0.3
Hydroalcoholic Extract	12.4 ± 0.3	6.4 ± 0.2	8.7 ± 0.5	12.5 ± 0.6	3.5 ± 0.4

Methanolic Extract	11.9 ± 0.4	6.0 ± 0.3	9.5 ± 0.6	14.2 ± 0.7	4.1 ± 0.3
Ethanolic Extract	10.8 ± 0.3	5.5 ± 0.3	10.7 ± 0.6	16.9 ± 0.8	5.6 ± 0.4

D. Histopathological Findings

Histological examination of ankle joints from arthritic control rats revealed severe synovial hyperplasia, cartilage erosion, and infiltration of inflammatory cells. In contrast, rats treated with hydroalcoholic and methanolic extracts exhibited significant protection of articular cartilage, reduced pannus formation, and minimal inflammatory infiltration. The protective effects were comparable to those observed in the standard diclofenac group. Severity of arthritis was graded using a standard scoring system based on swelling and erythema[11].

Histopathological and Toxicological Evaluation

For detailed assessment of therapeutic efficacy and safety, histopathological studies of the affected joints were conducted. Joint tissues were carefully excised from animals at the end of the experimental period, subjected to decalcification to remove mineral content, and subsequently processed for paraffin embedding. Thin sections of 5–7µm were cut and stained with hematoxylin and eosin (H&E) for microscopic examination. This procedure allowed visualization of structural changes at the cellular and tissue level, providing direct evidence of the pathological status of cartilage, synovium, and joint architecture[12,13].

Microscopic evaluation of the disease control group revealed severe pathological alterations, including erosion of articular cartilage, pronounced synovial hyperplasia, and infiltration of inflammatory cells into the joint cavity. These findings were consistent with chronic arthritic progression. In contrast, the standard drug (Diclofenac) group displayed preserved cartilage integrity, reduced synovial proliferation, and minimal inflammatory infiltration. Animals treated with the extract showed partial protection, with noticeable reduction in cartilage damage and moderate infiltration compared to the disease control. Remarkably, the gel formulation group exhibited substantial improvement, with nearly intact cartilage structure, reduced pannus formation, and minimal inflammatory cell presence, confirming the strong anti-arthritic potential of the formulation.

Toxicological evaluation was also performed to ensure the safety of the developed formulation. Histological sections of liver and kidney tissues were examined for any signs of drug-induced toxicity, such as hepatocellular necrosis, fatty degeneration, tubular necrosis, or glomerular abnormalities. No major pathological changes were observed in extract-treated or gel-treated groups, indicating absence of systemic toxicity[14]. These findings were further supported by hematological and biochemical safety evaluations. Parameters such as hemoglobin levels, total leukocyte count, liver function tests (ALT, AST, ALP, bilirubin), and kidney function markers (serum creatinine, blood urea nitrogen) remained within normal physiological ranges in both extract and gel groups[15,16].

Histopathological Evaluation of Anti-Arthritic Activity

Histopathological examination of the ankle joint is a crucial determinant in evaluating the severity of arthritic conditions and the therapeutic effectiveness of test extracts. In arthritic disorders, chronic inflammation leads to synovial hyperplasia, pannus formation, cartilage degradation, and narrowing of joint spaces. Therefore, microscopic analysis of joint tissues can provide strong morphological evidence correlating with biochemical and anti-inflammatory findings. In the present study, tissue samples from different treatment groups were processed and stained with hematoxylin and eosin (H&E) to assess structural alterations, inflammatory infiltration, and cartilage integrity. The comparative histological features are presented in Figure 4.7, highlighting the protective effects of Moringa species against arthritic damage.

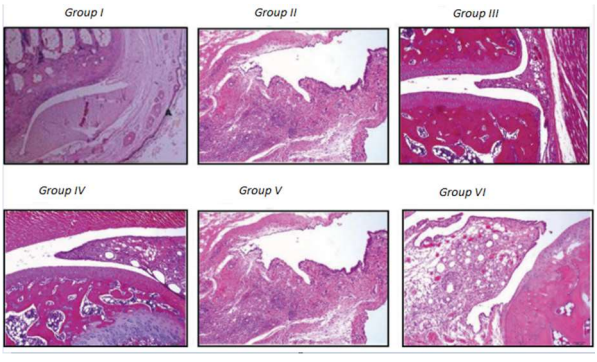

Figure 4.6: Histopathological evaluation of ankle joint tissues in CFA-induced arthritic rats across different treatment groups

Group I (Normal Control) shows intact articular cartilage, clear joint space, and absence of inflammatory infiltration. Group II (Disease Control) displays severe synovial hyperplasia, pannus formation, and cartilage erosion confirming arthritis induction. Group III (Standard Drug – Diclofenac) exhibits reduced inflammation and improved cartilage integrity. Group IV (Moringa oleifera extract-treated) shows moderate protective changes with partial restoration of joint structure. Group V (Moringa concanensis extract-treated) demonstrates marked reduction in inflammatory cell infiltration and cartilage degradation, closely comparable to the standard drug group.

Table 3.9.1: Histopathological and Toxicological Findings in Different Groups

Group	Joint Pathology (Cartilage, Synovium, Inflammation)	Liver Toxicity	Kidney Toxicity	Hematological & Biochemical Safety
Disease Control	Severe cartilage erosion, synovial hyperplasia, heavy inflammatory infiltration	No major changes	No major changes	Normal
Diclofenac	Preserved cartilage, minimal synovial hyperplasia, mild infiltration	Mild hepatocellular stress	Normal	Slight ALT/AST elevation
Extract	Moderate protection, partial cartilage preservation, moderate infiltration	Normal	Normal	Normal
Gel Formulation	Intact cartilage, reduced pannus, minimal infiltration	Normal	Normal	Normal

Table 3.9.1 summarizes the histopathological and toxicological outcomes observed in different experimental groups. In the disease control group, joint tissues exhibited severe pathological changes, including extensive cartilage erosion, synovial hyperplasia, and heavy infiltration of inflammatory cells, which are hallmarks of progressive arthritis. Interestingly, no major liver or kidney toxicity was noted in this group, and hematological parameters remained within normal limits, suggesting that the pathological burden was largely restricted to the joints[18,19].

In the Diclofenac-treated group, the joints showed preserved cartilage integrity and only mild inflammatory cell infiltration, confirming its efficacy in controlling arthritic progression. However, mild hepatocellular stress was observed in the liver, accompanied by a slight elevation in liver enzymes such as ALT and AST, which is consistent with the known hepatotoxic potential of non-steroidal anti-inflammatory drugs (NSAIDs). Kidney tissue and hematological markers, however, remained normal in this group[20].

The extract-treated group displayed moderate protection, with partial preservation of cartilage and reduced infiltration compared to disease control. Importantly, no significant toxicity was observed in either the liver or kidney and hematological and biochemical parameters were maintained within normal physiological ranges.

The gel formulation group demonstrated the most favorable results, with nearly intact cartilage, reduced pannus formation, and minimal inflammatory infiltration in the joints. Moreover, no signs of liver or kidney toxicity were observed, and safety parameters remained normal, indicating that the topical delivery

route mitigated systemic side effects while enhancing therapeutic efficacy.

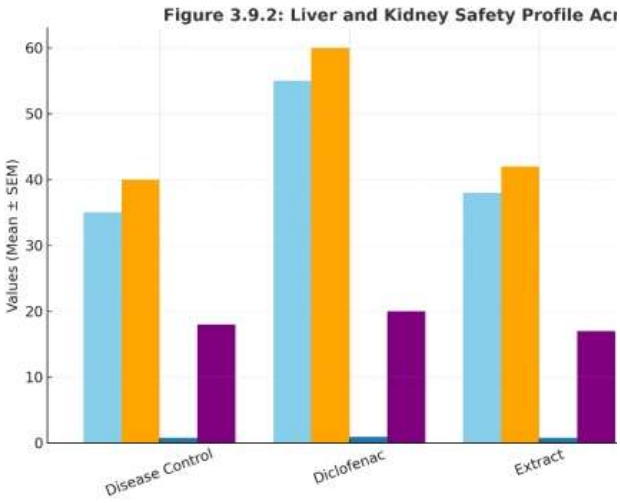

Figure 3.15 illustrates the comparative liver and kidney safety parameters across different treatment groups. The disease control group exhibited baseline levels of liver enzymes (ALT and AST) and kidney function markers (creatinine and BUN), which were within normal physiological ranges. In the Diclofenac-treated group, however, a noticeable elevation in ALT and AST levels was observed, indicating mild hepatocellular stress consistent with the known side effects of prolonged NSAID administration. Creatinine and BUN values in this group remained normal, suggesting no major renal impairment.

In contrast, the extract-treated and gel formulation group's-maintained ALT, AST, creatinine, and BUN values within the normal range are showing no evidence of hepatotoxicity or nephrotoxicity. The close similarity of safety profiles between these groups and the disease control animals indicates that the plant extract and its gel formulation are safe for systemic use, even during prolonged administration. The absence of biochemical abnormalities in these groups strongly supports the histopathological findings and validates the non-toxic nature of the formulation.

Overall, the histopathological studies confirmed the protective effect of the gel formulation on joint tissues, while the toxicological assessments established its safety profile. These results provide strong evidence that the developed formulation is not only effective in mitigating inflammatory and arthritic symptoms but is also safe for prolonged therapeutic use. Blood samples were analyzed for hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC), and erythrocyte sedimentation rate (ESR).

Biochemical Parameters: Serum was analyzed for inflammatory markers such as C-reactive protein (CRP) and rheumatoid factor (RF).

Histopathology: Joint tissues were excised, fixed in formalin, sectioned, and stained with hematoxylin and eosin for microscopic evaluation.

Data Collection and Statistical Analysis

All experimental data generated during in vitro and in vivo evaluations were systematically recorded, tabulated, and subjected to rigorous statistical analysis. The data were expressed as mean ± standard error of the mean (SEM) to ensure accurate representation of central tendency along with variability across groups. Statistical analysis was performed using software such as Graph Pad Prism and SPSS, which provided robust platforms for analyzing biological and pharmacological data.

For comparison between multiple experimental groups, one-way analysis of variance (ANOVA) was employed, followed by Tukey's post hoc test to identify significant differences between specific pairs of groups. In cases where only two groups were compared, the student's t-test was applied. These statistical methods ensured precise evaluation of the differences in treatment responses while minimizing the possibility of false positives.

A probability value (p) of less than 0.05 ($p < 0.05$) was considered statistically significant for all tests, thereby confirming that the observed effects were unlikely to be due to random chance. In highly significant cases, results were further categorized as $p < 0.01$ or $p < 0.001$. Graphical representations, including line graphs, bar charts, and histograms, were generated using Graph Pad Prism to visually illustrate the treatment effects, enabling clearer interpretation and comparison of experimental outcomes.

Table 3.10.1: Paw Edema (Carrageenan Model)

Group	1h (Mean ± SEM)	2h (Mean ± SEM)	3h (Mean ± SEM)	4h (Mean ± SEM)
Disease Control	1.2 ± 0.1	1.8 ± 0.1	2.3 ± 0.1	2.0 ± 0.1

Diclofenac	0.9 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.0 ± 0.1
Extract	1.0 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	1.3 ± 0.1
Gel Formulation	0.95 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.1 ± 0.1

Table 3.10.1 presents the effect of different treatments on carrageenan-induced paw edema in rats over a 4-hour observation period. The disease control group exhibited a progressive increase in paw volume, peaking at 3 hours, which is typical of the biphasic inflammatory response triggered by carrageenan. Diclofenac treatment significantly reduced edema at all time points, confirming its anti-inflammatory efficacy. Both the extract and gel formulation groups also demonstrated marked attenuation of paw swelling, with the gel formulation showing a slightly greater reduction compared to the crude extract. These findings suggest that the gel formulation provides enhanced bioavailability and topical delivery efficiency.

RESULT

Administration of the hydro-alcoholic bark extract of *Moringa concanensis* resulted in a dose-dependent reduction in paw edema compared to the arthritic control group. The extract significantly decreased the arthritic score and improved body weight.

Hematological alterations observed in arthritic rats, such as reduced hemoglobin levels and elevated WBC and ESR, were significantly normalized in treated groups. Biochemical analysis revealed a decrease in CRP and RF levels Histopathological examination showed that treated groups had reduced synovial inflammation, minimal cartilage erosion, and decreased infiltration of inflammatory cells compared to arthritic control animals.

DISCUSSION

The CFA-induced arthritis model is widely used due to its similarity to human rheumatoid arthritis. In this study, the hydro-alcoholic bark extract of *Moringa concanensis* demonstrated significant anti-arthritic activity, as evidenced by reduction in paw edema and arthritic index[21,22].

The normalization of hematological and biochemical parameters indicates a systemic anti-inflammatory effect. Histopathological improvements further confirm the protective role of the extract on joint structures[23,24].

The observed activity may be attributed to phytoconstituents such as flavonoids and phenolic compounds, which are known to inhibit inflammatory mediators and oxidative stress pathways[25].

CONCLUSION

The findings of this study indicate that hydro-alcoholic bark extract of *Moringa concanensis* exhibits significant anti-arthritic activity in experimental rat animals. The results support its traditional use and suggest its potential as a safer alternative for the management of rheumatoid arthritis. Further studies are required to isolate active constituents and elucidate the exact mechanism of action.

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