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RESEARCH ARTICLE OPEN ACCESS

Effect of Meloxicam and Nano-meloxicam on selected haematological and immunological parameters in arthritis-induced male albino rats

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Abstract
Objective: This study aimed to evaluate the effects of meloxicam and nano-meloxicam on haematological parameters and cytokine levels in an animal model of arthritis.
Methods: Forty Male albino rats were divided into four groups: Control, Arthritis, Arthritis + Meloxicam, and Arthritis + Nano-Meloxicam. Arthritis was induced by injecting Complete Freund's adjuvant into the foot pad of albino rats at a dose of 0.1 ml for 14 days. Both Meloxicam and Nano-Meloxicam were administered at doses of 0.6 mg/kg bw for 30 days. Haematological markers, including WBC count, RBC count, haemoglobin, PCV and platelet count, were measured. The differential leukocyte count, consisting of lymphocytes, neutrophils, monocytes, and eosinophils, was analysed. Pro-inflammatory cytokine levels, such as IL-1, IL-6, and TNF-α, were quantified. The one-way ANOVA test was used for comparison, and Fisher's Least Significant Difference was derived. The p-value was set at 5%.
Results: The arthritis group showed a significant increase in WBC count and a decrease in RBC count, haemoglobin, and packed cell volume, along with an elevated platelet count. This group also presented discrepancies in the differential leukocyte count and higher concentrations of IL-1, IL-6, and TNF-α. Treatment with meloxicam and nano-meloxicam led to the restoration of haematological parameters and differential leukocyte counts and reductions in pro-inflammatory cytokine levels. The Arthritis + Nano-Meloxicam group demonstrated the most beneficial outcomes
Conclusion: The study suggests an enhanced therapeutic efficacy of nano-formulation in addressing the haematological and immunological abnormalities associated with rheumatoid arthritis, highlighting the therapeutic value of nano-meloxicam in treating rheumatoid arthritis.

Keywords: Rheumatoid arthritis, Haematological parameters, Cytokines, Meloxicam, Nano-meloxicam

Plain English Summary
Arthritis is a long-term inflammatory disease that can be caused by blood and immune system abnormalities. This study assessed the effects of a nonsteroidal anti-inflammatory drug (meloxicam) on blood parameters and signalling protein levels in an animal model of arthritis. We used male albino white rats which were divided into four experimental groups. The arthritis group showed an increase in white blood cell counts, and a decrease in red blood cell count, haemoglobin, and packed cell volume, suggesting a shortage of blood and altered immunological responses. Our study highlighted the therapeutic value of nonsteroidal anti-inflammatory drugs in treating arthritis.

Background

Arthritis refers to over 100 joint conditions that cause inflammation of one or more joints. Rheumatoid arthritis (RA), is a specific type of arthritis characterized by a long-term autoimmune condition that mostly affects the

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joints, resulting in stiffness, pain, and inflammation. The skin, eyes, lungs, heart, and blood vessels are among the other bodily systems that it may affect, is a common and debilitating joint disorder that affects millions of people worldwide, especially among the elderly. It is characterized by the progressive degeneration of articular cartilage, accompanied by inflammation, pain, and impaired joint function (1). The prevalence of RA is expected to increase due to the ageing population and rising rates of obesity and physical inactivity (2). Current pharmacological treatments for RA, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen, often provide only partial relief and are associated with various adverse effects, including gastrointestinal, cardiovascular, and renal complications (3).

One promising approach is the use of nanomedicine, which involves developing drug delivery systems at the nanoscale. Nanoparticles have the potential to enhance the pharmacokinetic and pharmacodynamic properties of drugs, improving their therapeutic efficacy and reducing side effects (4). Meloxicam, a selective COX-2 inhibitor, is widely used in managing RA due to its potent anti-inflammatory and analgesic properties (5). However, the oral administration of meloxicam is linked to gastrointestinal side effects, such as ulcers and bleeding (6). In this context, a recent study by Jyothi et al. (7) investigated the effects of nano-meloxicam in reducing symptoms associated with induced arthritis in male albino rats. A study by Nabizadeh et al. (8) evaluated the efficacy of a nano-formulation of celecoxib, another NSAID, using a rabbit model of anterior cruciate ligament transection-induced RA. The nano-celecoxib group showed improved joint function, reduced cartilage damage, and lower levels of inflammatory markers compared to the conventional celecoxib group. The study by Jyothi et al. (7) highlights the promising potential of nano-meloxicam in mitigating the effects associated with induced arthritis in male albino rats. The nano-formulation demonstrated superior efficacy in enhancing joint function, reducing inflammation, and attenuating cartilage degeneration compared to conventional meloxicam.

This study aimed to evaluate the effects of meloxicam and nano-meloxicam on haematological parameters and cytokine levels in an animal model of arthritis.

Materials and Methods

Experimental Animals

The study was conducted in the animal house of the Department of Life Sciences at the Faculty of Education, Al-Qadisiyah University, from September 2, 2023, to March 5, 2024. Male Albino rats were used in this study, which was purchased from the animal house of the Faculty of Veterinary Medicine, Al-Qadisiyah University. The rats were raised to ensure similar ages and weights for the experiment, with weights ranging from 200 to 250 grams and ages between 3 to 4 months. The experimental animals were housed in special plastic cages prepared for this purpose, which had metal mesh lids, a specialized water drinking system, and bedding made of sawdust. The cages were kept clean and sterilized with disinfectants. The experimental animals were maintained under suitable laboratory conditions at a temperature of 20 to 25°C.

Induction of Arthritis

Complete Freund's adjuvant was used to induce the development of arthritis, which was purchased from (Sigma Aldrich). After dissolving the daily dose of Complete Freund's adjuvant, each animal was injected daily into the right foot pad using an insulin syringe for 14 days, and random blood samples were drawn to confirm the development of arthritis. The results showed an increase in the level of rheumatoid factor and anti-CCP.

Meloxicam Drug

Meloxicam drug was used in this study, which was obtained from a registered pharmacy in Diwaniyah city. The dose was 0.6 mg/kg body weight of Meloxicam drug, and after dissolving the full daily dose of the drug in distilled water, each animal was dosed daily with 1 mL orally using a special wine syringe for this purpose containing a hooked needle.

Preparation of the zinc oxide nano-loaded drug Meloxicam

Nano-loaded anti-arthritic drug Meloxicam at a concentration of 0.6 mg/kg body weight was loaded onto zinc oxide which was used at a concentration of 50 mg/kg body weight (9). After meloxicam was powdered using an electric grinder and sieved to obtain a fine powder, meloxicam was loaded onto zinc oxide in several sequential steps. First, 1.2 grams of meloxicam was dissolved in water and then dissolved 1.2 grams of zinc oxide. Subsequently, 1.2 g of the drug was dissolved in 2.4 mL of Di Methyl Sulfate (DMSO) and MnOxide and then the volume was completed to 50 ml using deionized water. Secondly, 1 g of zinc oxide was dissolved in 50% Absolute methanol. Thirdly, 50 mL of the drug prepared in Step 1 was added in successive drops to 50 ml of the zinc oxide solution prepared in Step 2. In the fourth step, the mixture prepared in step 3 was stirred for two hours on a magnetic stirrer and then placed in a shaking incubator at

37°C for 18 hours. Subsequently, the mixture was placed in the static incubator at 40°C for another 24 hours, after which the precipitate was separated using a centrifuge at 5000 rpm for 20 minutes. The precipitate was then washed several times with deionized water and each time it was washed, it was separated by centrifugation at 3000 rpm for 15 minutes. Finally, the precipitate was dried at 40°C, ground in a ceramic mortar and finally stored.

Study Design

In this experiment, 40 male rats were used and randomly divided into four groups with 10 rats per group. The animals were divided as follows- **C: Control group:** They were fed with the serum solution during the duration of the experiment. **T1:** Animals in which arthritis is induced by injecting Complete Freund's adjuvant into the foot pad of albino rats at a dose of 0.1 ml for 14 days. **T2:** Animals in which arthritis is induced for 14 days and then administered meloxicam at a dose of 0.6 mg/kg bw for 30 days. **T3:** Group of animals in which arthritis is induced for 14 days and then administered Nano-meloxicam at a dose of 0.6 mg/kg bw for 30 days.

Statistical Analysis

The results of the experiments were analysed using SPSS statistical software. A one-way ANOVA test was used to compare the studied groups with the control group and Least Significant Differences (LSD) and p-values (5%) were calculated to test the significance of the results.

Results

Blood Parameters

The Arthritis group displayed a significant increase in WBC count (20.394 ± 0.305 × 10^3/µl) compared to the Control group (8.713 ± 0.086 × 10^3/µl). Treatment with meloxicam (Arthritis + Meloxicam group: 15.327 ± 0.189 × 10^3/µl) and nano-meloxicam (Arthritis + Nano-Meloxicam group: 14.078 ± 0.163 × 10^3/µl) led to a decrease in white blood cell count, with the Arthritis + Nano-Meloxicam group achieving the best outcome. The obtained LSD value of 0.421 confirms the statistical significance of the differences among the groups. The Arthritis group also showed a significant reduction in RBC count (4.249 ± 0.020 × 10^6/µl) in comparison to the Control group (8.298 ± 0.035 × 10^6/µl), suggesting the presence of anaemia. The findings were statistically significant with p-values <0.05. See Table 1.

Treatment with meloxicam (Arthritis + Meloxicam group: 5.109 ± 0.046 × 10^6/µl) and nano-meloxicam (Arthritis + Nano-Meloxicam group: 5.700 ± 0.049 × 10^6/µl) resulted in increased RBC count, with the Arthritis + Nano-Meloxicam group. The reported LSD value of 4.14 validates the statistical significance of the differences among the groups.

Table 1. Effect of both meloxicam and Nano-meloxicam on haematological parameters in arthritis-induced male albino rats.

Groups	WBCs $X10^3$/µl	RBCs $X10^6$/µl	HB g/dl	PCV %	Platelet (10^3UI)
C	8.71 ±0.086^G	8.29 ±0.035^A	14.05 ±0.10^A	42.17 ±0.30^A	854.33 ±4.322^F
T1 (Arthritis)	20.39 ±0.305^A	4.24 ±0.020^F	5.52 ±0.059^F	16.56 ±0.179^G	1229.56 ±3.778^A
T2 (Arthritis+ meloxicam)	15.32 ±0.189^C	5.10 ±0.046^E	7.35 ±0.120^D	22.06 ±0.361^E	984.00 ±2.892^C
T3 (Arthritis + nano- meloxicam)	12.07 ±0.163^E	5.70 ±0.049^D	8.59 ±0.112^C	26.79 ±0.337^D	965.49 ±18.725^D
LSD	0.421	0.215	0.201	1.124	10.14
p-value 5%					

Values represent mean ± standard error. Similar letters between any two groups indicate no significant differences while different letters indicate significant differences at (p<0.05). C: Control, T1: CJ arthritis induction, T2: Induction of CJ arthritis with Meloxicam, T3: Induction of CJ arthritis with Nano-Meloxicam.

Cytokines (IL1-, IL-6, TNF-α)

The arthritis group exhibited a significant increase in IL-1 levels (152.65 ±4.195 pg/ml) compared to the Control group (25.69 ±0.677 pg/ml) The administration of meloxicam (Arthritis + Meloxicam group) and nano-meloxicam (Arthritis + Nano-Meloxicam group) resulted in a reduction in IL-1 levels, with the Arthritis + Nano-Meloxicam group (84.94 ±9.527 pg/ml) showing the most favourable outcome compared to the Arthritis + Meloxicam group (94.88 ±3.876 pg/ml). The reported LSD value of 1.341 confirms the statistical significance of the differences between the groups. The arthritis group had significantly elevated IL-6 levels (134.51±4.336 pg/ml) compared to the Control group (46.34 ±4.56 pg/ml), indicating the role of this pro-inflammatory cytokine in the arthritic condition.

The Arthritis + Meloxicam (95.40±8.447 pg/ml) and Arthritis + Nano-Meloxicam (81.88±6.180 pg/ml) groups showed a decrease in IL-6 levels, with the Arthritis + Nano-Meloxicam group demonstrating the most pronounced improvement. The reported LSD value of 1.147

suggests the statistical significance of the observed differences. The Arthritis group had a significant increase in TNF-α levels (138.51 ±4.759 pg/ml) compared to the control group (32.19 ±3.084 pg/ml), suggesting the involvement of this cytokine in the inflammatory processes associated with the arthritic condition. Both the Arthritis + Meloxicam (82.07±3.731 pg/ml) and Arthritis + Nano-Meloxicam (72.87±9.473 pg/ml) groups demonstrated a reduction in TNF-α levels, with the Arthritis + Nano-Meloxicam group exhibiting the most favourable outcome. The reported LSD value of 1.017 confirms the statistical significance of the differences between the groups. The statistical significance of the differences between the groups, as indicated by the reported LSD values, underscores the reliability and potential clinical relevance of the observed effects.

Table 2. Effect of meloxicam and nano-meloxicam and nanoscale zinc on cytokine levels in arthritis-induced male albino rats.

Groups	IL-1 Pg/ml	IL-6 Pg/ml	TNF-a Pg/ml
C	45.69±0.677^{G}	46.34±4.56^{F}	32.19±3.084^{G}
Arthritis	152.65±4.195^{A}	134.51±4.336^{A}	138.51±4.759^{A}
Arthritis+ meloxicam	94.88±3.876^{C}	95.40±8.447^{C}	82.07±3.731^{C}
Arthritis + nano-meloxicam	84.94±9.527^{D}	81.88±6.180^{D}	72.87±9.473^{D}
LSD	1.341	1.147	1.017
p-value 5%			

Values represent mean ± standard error. Similar letters between any two groups indicate no significant differences while different letters indicate significant differences at (p<0.05). C: Control, T1: CJ arthritis induction, T2: Induction of CJ arthritis with Meloxicam, T3: Induction of CJ arthritis with Nano-Meloxicam.

Discussion

Blood Parameters

The findings of the present study indicated that the induction of arthritis in the Arthritis group led to significant alterations in many haematological parameters compared to the control group. The changes observed in the haematological parameters of the arthritis group, including elevated WBC count, reduced RBC count, decreased Hb levels, and lowered PCV, suggest that anaemia and altered immune responses are associated with rheumatoid arthritis (10, 11).

The increased platelet count in the arthritis group may reflect the inflammatory nature of the disease. The administration of meloxicam and nano-meloxicam has demonstrated beneficial effects on the haematological parameters in the arthritic model. The improvements seen in the Arthritis + Nano-Meloxicam group can be attributed to the superior anti-inflammatory and restorative properties of the nanoparticle formulation, which may have aided in alleviating haematological abnormalities (12, 13). The heightened positive effects observed in the Arthritis + Nano-Meloxicam group compared to the Arthritis + Meloxicam group can be linked to the enhanced pharmacokinetic and pharmacodynamic properties of the nanoparticle formulation, resulting in more effective modulation of inflammatory and haematological responses. The statistical significance of the differences between the groups, as indicated by the reported LSD values, emphasizes the reliability and potential therapeutic relevance of the observed effects.

Cytokines (IL-1, IL-6, TNF-α)

The arthritis group had markedly increased levels of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α, in contrast to the control group. The increased concentrations of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α, identified in the arthritis group align with the recognized inflammatory mechanisms underlying rheumatoid arthritis (14, 15). These cytokines are essential in sustaining the inflammatory cascade, resulting in joint destruction and further systemic signs of the illness. The injection of meloxicam, a selective COX-2 inhibitor, and nano-meloxicam, a nanoparticle formulation of meloxicam, had advantageous effects on the levels of pro-inflammatory cytokines in the arthritic animal. The enhancements noted in the Arthritis + Nano-Meloxicam cohort can be ascribed to the increased bioavailability and targeted administration of the medication, perhaps resulting in more effective control of inflammatory pathways (13, 16).

The enhanced beneficial effects noted in the Arthritis + Nano-Meloxicam group relative to the Arthritis + Meloxicam group can be ascribed to the superior pharmacokinetic and pharmacodynamic characteristics of the nanoparticle formulation, potentially leading to more effective inhibition of pro-inflammatory cytokines (12). The statistical significance of the group differences, as evidenced by the reported LSD values, highlights the dependability and possible therapeutic importance of the observed effects.

Conclusion

The study findings indicate that the production of arthritis in the animal model resulted in notable haematological and immunological disruptions, marked by changes in leukocyte count, RBC parameters, and pro-inflammatory cytokine levels. The administration of meloxicam, particularly in its nano-formulation, had advantageous effects in alleviating these abnormalities, underscoring the prospective therapeutic benefits of nano-formulation in the treatment of rheumatoid arthritis.

List of Abbreviations

WBC: White blood cells
RBC: Red blood cells
PCV: Packed cell volume
RA: Rheumatoid arthritis
NSAIDs: Nonsteroidal anti-inflammatory drugs
DMSO: Dimethyl Sulfate
LSD: Least Significant Differences

Declarations

Ethical approval and consent to participate

This research was sanctioned by the Scientific Research Ethics Committee of the Department of Biology, Faculty of Education, Qadisiyah University. All experiments were performed following international best ethical practices.

Consent for publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution- Non-Commercial 4.0 license.

Availability of data and materials

The data and materials associated with this research will be made available by the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Nil.

Author contributions

AKB was responsible for all aspects of the research, including conceptualization, data collection, laboratory analysis, and manuscript draft. WMK was responsible for research conceptualization, data collection and laboratory analysis. All the authors confirmed the final version and assented to the manuscript's publication.

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