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The potential of methotrexate-loaded nanoparticles to alleviate autoimmune arthritis in the clinical context of a urinary tract infection

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SUMMARY

AUTHORS' CONTRIBUTION: (A) Study Design · **(B)** Data Collection. **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** Funds Collection

Background: Rheumatoid Arthritis (RA) is a progressive inflammatory illness marked by inflammation and overgrowth of the synovial membrane, leading to the creation of rheumatoid pannus.

Method: 125 urine samples and blood were obtained from individuals diagnosed with RA who sought medical attention at the general hospital in the province between the dates of 2 January and 14 February 2024. Blood sample was drawn for the purpose of conducting a series of immune tests, including those for IgG, IgM, IL-2, IL-4, IL-13, TNF-α, and TLR2 and TLR4. Vitro study employed a total of 50 rats, consisting 25 males and 25 females. The rats were randomly assigned to one of four groups, The sham group was supplied physiological saline (0.9%), whereas the control/ induction group got an intradermal injection 100 µg of CII emulsified. The MTX group was treated with methotrexate (MTX). The group treated with nano-asparagine solely comprised rats that were treated with MTX.

Result: Mean age and BMI were 31 and 23. Participants aged >50 years accounted for 46%, with 39% classified as obese per WHO standards. Most participants were female (68%). There were 47 patients (37.67%) with E. coli and 28 patients (22.33%) with Klebsiella pneumoniae. At the probability level ($P<0.05$), the current study's findings, which aimed to identify the presence of tumor necrosis factor alpha, interleukin-2, interleukin-4, interleukin-13, toll-like receptor 2, and toll-like receptor 4, showed statistically significant differences. The % in female patients was significantly greater than the percentage in male patients, reaching 1.23 ± 0.033, 1.15 ± 0.58, 1.069±0.236, 1.532 ± 0.173, 0.90 ± 0.022, and 0.96 ± 0.018 pg/ml, respectively. The mean results for the patients were 0.37 ± 0.01, 0.27 ± 0.02, 0.4831 ± 0.0528, 0.4259 ± 0.0554, 0.5187 ± 0.0950, and 0.4748 ± 0.0776 pg/ml, respectively.

Conclusion: Our investigation shows MTX-NPs effectively treat CIA in rats. Subcutaneous MTX-NPs reduced arthritis progression, inflammatory cell infiltration, and inflammatory factor levels, highlighting their potential as an advanced alternative to standard MTX therapy.

Keywords: Autoimmune arthritis; Urinary tract infection; Nano-particles

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INTRODUCTION

Rheumatoid Arthritis (RA) is a progressive inflammatory disease typified by synovial membrane inflammation and overgrowth that result in rheumatoid pannus. Degeneration of adjacent cartilage and bone tissues follows from this process. RA's pathogenesis is caused by a complex interaction of inflammatory molecules including prostanoids, proteolytic enzymes, and cytokines and immune cells [1,2]. Although the exact origins of rheumatoid Arthritis (RA) are yet unknown, autoantibodies and inflammatory cytokines like tumor necrosis factor (TNF-α), interleukin (IL)-6, IL-1β, and IL-13 are produced by T and B cell invasion into the joints. This mechanism promotes the growth of synoviocytes resembling fibroblast and causes bone breakdown [3].

Methotrexate (MTX) is a first-line Disease-Modifying Antirheumatic Drug (DMARD) that has been demonstrated to reduce joint deterioration in patients with rheumatoid arthritis (RA) [4,5]. It is an antifolate metabolite that inhibits folate-dependent enzymes in the de novo synthesis of purines and pyrimidines [6]. MTX is administered as monotherapy in patients with early RA [7] and as an anchor medicine for combination therapy with other DMARDs or biologics in patients with established RA who do not respond successfully to MTX. However, long-term usage of MTX has been observed to result in drug resistance and the production of substantial side effects, including nausea, neutropenia, pulmonary fibrosis and hepatitis [8].

Nanoparticles (NPs) have the potential to be a very promising class of therapeutics due to their capacity to distribute and release drugs in a regulated manner [9]. A number of nanoparticles have been produced for the objective of drug delivery in the treatment of a variety of illnesses. They can impede the fast removal of drugs from the body by methods of delayed release. Nanoparticles are frequently administered via intravenous injection, oral feeding, or subcutaneous injection. Intravenously delivered NPs are efficacious but also rapidly removed, thereby boosting the possibility of side consequences [10]. The most basic and low-risk technique is oral feeding; unfortunately, the absorption rate is insufficient. Subcutaneous injection demonstrates intermediate efficacy in contrast to other techniques, thereby presenting an advantage in occupying a medium ground. Nevertheless, the bulk of NP study for RA therapy have

included intravenous injection and oral feeding [11].

METHODS

The process of collecting and identifying bacterial isolates is described below. In the present study, 125 urine samples and blood were collected from patients diagnosed with RA who sought medical attention at the general hospital in the province between the dates of 2 January and 14 February 2024. The study population consisted of patients diagnosed with Rheumatoid Arthritis (RA) in line with the ACR/EULAR classification criteria for RA.

Patients with rheumatoid arthritis must be at least 16 years of age, have not utilized other Disease-Modifying Antirheumatic Medications (DMARDs) within 12 weeks of screening, and have poor control of rheumatoid arthritis symptoms when on Methotrexate (MTX) treatment. The presence of two or more swollen joints, or five or more swollen and painful joints, in association with an Erythrocyte Sedimentation Rate (ESR) of 10 mm/h or greater.

The study excluded Rheumatoid Arthritis (RA) individuals with thyroid or parathyroid illness, or those who were using medicines for these diseases. Additionally, patients with an acute or recent infection, contraindications for methotrexate or anti-tumour necrosis factor drugs, chronic renal or liver failure, osteoporosis prior to the diagnosis of the disease, other autoimmune disorders, bone or bone marrow diseases, and chest infections during therapy were excluded. The study did not include pregnant women or lactating mothers.

Urine sample collection: All urine samples were subjected to standard bacteriological processes and characterized based on their culture morphology and microscopic examination, as well as biochemical tests. The identification of the clinical isolates was confirmed via the automated Vitek-II system (Biomerieux, France).

Blood sample collection: A total of 5 ml of blood was drawn for the purpose of conducting a series of immune tests, including those for IgG, IgM, IL-2, IL-4, IL-13, TNF-α, and TLR2 and TLR4. The samples were then placed in blood separation tubes and gel tubes, and subsequently centrifuged at a speed of 3000 rpm for 3 minutes, with the resulting serum then obtained. Subsequently, the serum was transferred to new white tubes and maintained at a temperature of (-20), whereupon it was employed in the conduct of immune tests.

Preparation of MTX nano-particles: Poly (lactic-co-glycolic acid) (50 mg) and methotrexate (5 mg) were dissolved in DMSO at 60°C to create methotrexate-loaded nanoparticles. Dropwise additions were made to 1% PVA (w/v aqueous solution). The solution was homogenized for two minutes at 7000 RPM using an Ultra Turrax® T-25 homogenizer (IKA®-Werke, Staufen, Germany). Unencapsulated compounds were dialyzed in distilled water for an hour using a 14,000 molecular weight cut-off membrane. The MTX-NP size distribution was determined by measuring in PBS with a Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK) at 25°C. Measure MTX absorbance at 370 nm to measure the amount of encapsulated MTX; calculate encapsulation efficiency with this formula: Dividing the encapsulated medicine by the extra drug yields a 100-fold figure. MTX-NP was immersed in 50 mL PBS in a dialysis bag to assess drug release. MTX absorbance at 370 nm was determined using a microplate reader (Synergy H1, Bio-Tek, USA) after removing 200 µL of external PBS solution at preset intervals.

CIA induction: The study involved a total of 50 rats, comprising 25 males and 25 females. The rats were of the Wistar strain and were aged between five and seven months, with an average weight of 165 to 175 grams. The rats were randomly assigned to one of four groups, with each group comprising five rats, and were subjected to two distinct treatment periods. The groups were treated as follows: The sham group was administered physiological saline (0.9%), while the control/induction group received an intradermal injection at the base of the tail with 100 µg of CII emulsified in Freund's adjuvant (Chondrex). The MTX group was treated with 2.5 mg/kg MTX twice weekly. The group treated with nano-asparagine only comprised rats that were treated with MTX. The MTX group comprised rats that were treated with 2.5 mg/kg MTX-NPs twice weekly.

Statistical analysis: All statistical analyses were conducted using Prism (version 8 for Windows; GraphPad Software). P-values were calculated using a two-tailed paired t-test and a two-way analysis of variance (grouped). A p-value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Demographic and clinical characteristics

The clinical demographic characteristics of the patient group are presented in Tab. 1. The mean age and BMI were 31 and 23, respectively. The results demonstrated that the highest percentage of age groups was 46% for the age range of >50 years. The BMI group of the participants was divided according to the WHO classification, with 39% of the participants falling within the obese range. Finally, the majority of participants were female (68%).

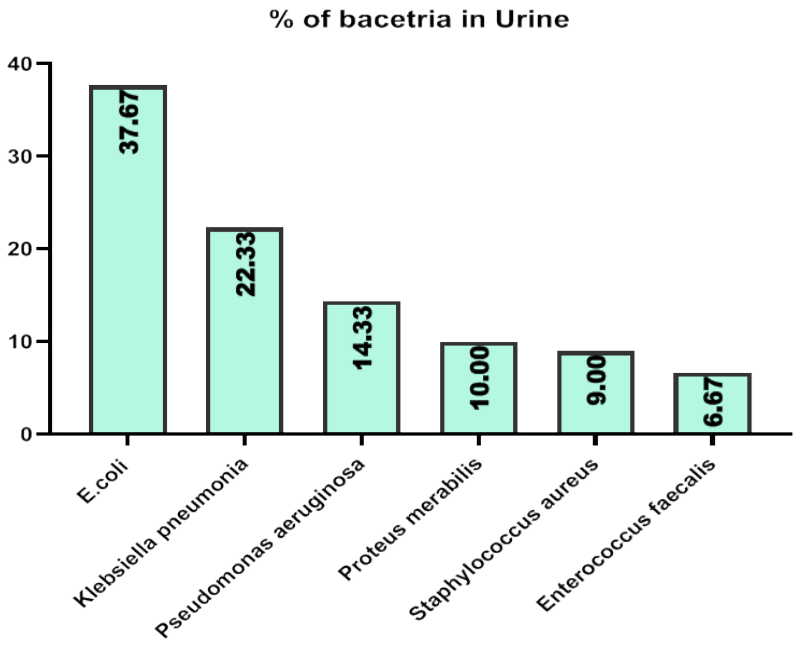
Tab. 1. Distribution of the samples according to demographic information (N=125).

Variables	Groups	N (%)
Gender	Male	40 (32%)
	Female	85 (68%)
Age Group (Years)	< 20	10 (8%)
	20-30	35 (28%)
	31-50	34 (27%)
	>50	46 (37%)
BMI kg/m^2	Under weight	9 (7%)
	Normal weight	17 (14%)
	Over weight	25 (20%)
	Obesity	39 (31%)

Distribution of UTI patients according to microorganism type

The distribution of samples according to microorganism type in the current study revealed that 47 patients (37.67%) had E. coli isolates, 28 patients (22.33%) had Klebsiella pneumoniae isolates, and 18 patients (41.3) the remaining isolates were identified as Pseudomonas aeruginosa (3%), Proteus mirabilis (10%), Staphylococcus aureus (9%), and Enterococcus faecalis (6.67%), as illustrated in Fig. 1.

Fig. 1. Distribution of UTI patients according to microorganism type.



Distribution of immunological factors

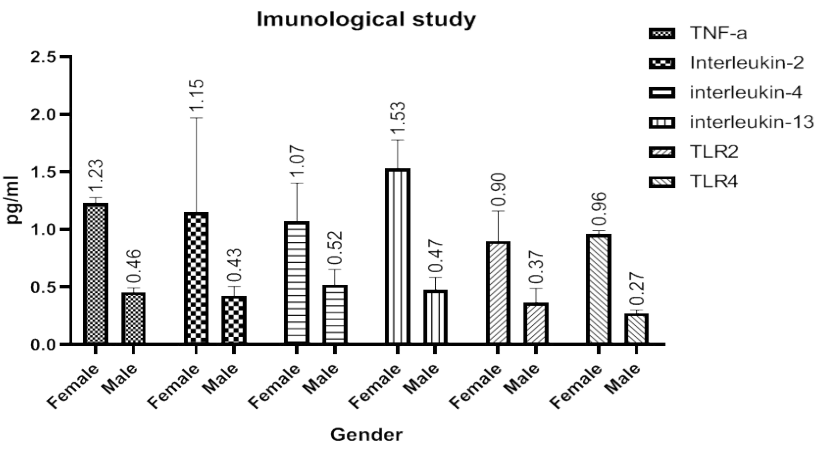
The results of the current study, which aimed to detect tumor necrosis factor alpha, interleukin-2, interleukin-4, interleukin-13, TLR2, and TLR4 in patients with rheumatoid arthritis, demonstrated statistically significant differences at the probability level ($P<0.05$). The percentage in female patients reached (1.23 ± 0.033, 1.15 ± 0.58, 1.06 9 ± 0.236, 1.532 ± 0.173, 0.90 ± 0.022, 0.96 ± 0.018, respectively) pg/ml, which was significantly higher than the percentage in male patients, the mean values were 0.4831 ± 0.0528, 0.4259 ± 0.0554, 0.5187 ± 0.0950, and 0.4748 ± 0.0776 pg/ml, 0.37 ± 0.01, 0.27 ± 0.02, respectively. Fig. 2. illustrates the results.

MTX-NPs decrease the severity of arthritis

The objective was to explore whether MTX-NPs could affect the development of an experimental model of arthritis in vivo. To this purpose, MTX or MTX-NPs were administered to rats with CIA at three weeks following CII immunization. The results demonstrated that the subcutaneous injection of MTX-NPs in arthritic rats led to a large reduction in the arthritis score and incidence in compared with vehicle-treated CIA rats.

Additionally, the levels of inflammatory mediators, including tumor necrosis factor alpha, interleukin-2, interleukin-4, interleukin-13, toll-like receptor 2, and toll-like receptor 4, were markedly reduced in the serum of MTX-NPs-treated rats in comparison to those treated with the vehicle (Fig. 3.).

Fig. 2. Levels of the immunological parameters in individuals in RA according to gender, TNF-a: Tumor necrotic alpha, TLR2: Toll like receptor-2, TLR4: Toll like receptor-4.



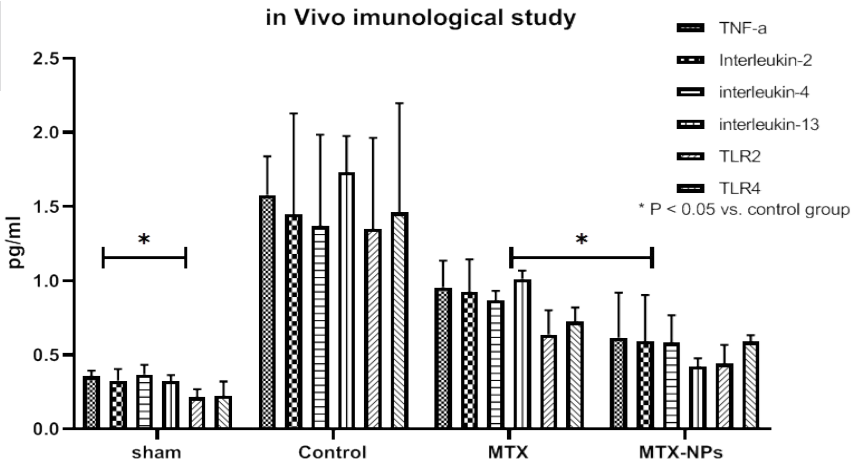


Fig. 3. MTX-NPs were reported to reduce the levels of inflammatory mediators *in vivo*.

DISCUSSION

The data indicated that Escherichia coli was the most prevalent bacterium responsible for urinary tract infections (UTIs), followed by Klebsiella pneumoniae and Pseudomonas aeruginosa. The bulk of investigations have suggested that Escherichia coli is the leading causal agent of UTIs, followed by Klebsiella pneumoniae. This agrees with our findings, which may provide a more realistic portrayal of the bacterial groups involved. In a study conducted by, the most prevalent bacteria were found to be Escherichia coli, followed by Klebsiella pneumoniae, then Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, and Enterococcus spp., with variations in their sequence of frequency [12,13].

The present study demonstrates that the levels of immunological parameters are elevated in female and control groups in comparison to male gender and MTX-NPs. The rate of female individuals reached (0.103 ± 0.201) pg/ml, which is consistent with the findings of previous studies [14] and also aligns with the results of researchers [10] who also observed an increase.

The level of tumor necrosis factor in female patients with Rheumatoid Arthritis (RA) was found to be higher than that in male patients, a result that is consistent with the findings of [15], who demonstrated that infection with RA leads to a rise in the production of TNF-α. This is related to the fact that macrophages create Nitric Oxide (NO), which assists them to eliminate inflammation. Additionally, tumor necrosis factor plays a role in increasing the INF-γ/Th1 response [16].

The function of this interleukin in the setting of RA is of great importance, as its increase has a substantial influence on the immunological response of T cells Th1, which safeguard the body. Additionally, low levels of IL have been connected to illness exacerbation, as indicated by the fact that some individuals with low IL levels had disease start and progression [17]. Additionally, this interleukin plays a role in the immune system's protective strategy by stimulating phagocytic cells and improving their capacity to engulf joints. Furthermore, this interleukin interacts with other cytokines, such as TNF-α and IL, in the establishment of an efficient immune response. High quantities of this interleukin have been linked to improved clinical outcomes in Rheumatoid Arthritis (RA) [18].

TLR receptors are the most raised in RA and are released predominantly by macrophages, also suggested that an increase in the level of TLR-2 TLR4 leads to a worsening of the illness due to boosting Th1 to differentiate and create TNF-α and other cytokines [19]. Additionally, a study conducted by the same author demonstrated that a drop in these receptors leads to an increase in the recovery rate [20]. It is obvious that TLR4 and TLR2 receptors have a role in the generation of tumour necrosis factor. Consequently, the neutralisation of these receptors results in a reduction in the production of these cytokines and an alteration in the immunological response [20].

CONCLUSION

Our findings demonstrate the therapeutic efficacy of MTX-NPs in rats with CIA. Subcutaneous therapy with MTX-NPs significantly slowed the course of arthritis in the experimental model and reduced the infiltration of cells expressing inflammatory markers. Furthermore, the levels of inflammatory factors were much lower in the MTX-NPs-treated group. These findings suggest that MTX-NPs could be a viable enhanced treatment technique for resolving the limits of conventional MTX therapy mated.

RECOMMENDATION

We propose to work on the efficacy of methotrexate as an antibacterial agent in rats and to complete the trial, as there were some issues that hindered the completion of the study.

REFERENCES

1. **Tu J, Huang W, Zhang W, et al.** Two main cellular components in rheumatoid arthritis: Communication between T cells and fibroblast-like synoviocytes in the joint synovium. *Front Immunol.* 2022;13:922111.
2. **Abbas Al-Huesini LM, Al-Mudhaffer RH, Hassan SM, et al.** DMF Ameliorating Cerebral Ischemia/Reperfusion Injury in Meal Rats. *Sys Rev Pharm.* 2019;10(1).
3. **McInnes IB, Schett G.** The pathogenesis of rheumatoid arthritis. *N Engl J Med.* 2011;365(23):2205-2219.
4. **Jura-Półtorak A, Szeremeta A, Olczyk K, et al.** Bone metabolism and RANKL/OPG ratio in rheumatoid arthritis women treated with TNF-α inhibitors. *J Clin Med*. 2021;10(13):2905.
5. **Hassan SM, Al-Jaf AN, Hussien YA, et al.** Effect of new synthesized

compounds of 2-Thiouracil Sulfonamide Derivatives against colon and liver carcinoma cells "*In Vitro* Study. *Inter J Pharm Res.* 2020;12(4):2012-2016.

6. **Cronstein BN, Aune TM.** Methotrexate and its mechanisms of action in inflammatory arthritis. *Nat Rev Rheumatol.* 2020;16(3):145-154.
7. **Friedman B, Cronstein B**. Methotrexate mechanism in treatment of rheumatoid arthritis. *Joint Bone Spine.* 2019;86(3):301-307.
8. **Mei L, Gao K, He X, et al.** Disease-modifying antirheumatic drugs: Approaches and lessons learned from traditional medicine. *Front Pharmacol.* 2023;14:1135803.
9. **Yoon HY, Koo H, Choi KY, et al.** Photo-crosslinked hyaluronic acid nanoparticles with improved stability for *in vivo* tumor-targeted drug delivery. *Biomater*. 2013;34(21):5273-5280.
10. **Hassan SM, Obeid HA, Hasan IS, et al.** Etanercept ameliorated cerebral damage during global cerebral ischemia-reperfusion injury in male rats. *Azerbaijan Pharma Pharmacother J.* 2023;22(1):53-58.
11. **Thambi T, Deepagan VG, Yoon HY, et al.** Hypoxia-responsive polymeric nanoparticles for tumor-targeted drug delivery. *Biomater.* 2014;35(5):1735-1743.
12. **Jalil MB, Al Atbee MY.** The prevalence of multiple drug resistance *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients with urinary tract infections. *J Clin Lab Anal.* 2022;36(9):e24619.
13. **Hussein ZA, Yousif J.** Use of *Citrullus colocynthis* fruits and *Quercus* spp. bark extracts as scolicidal agents for protoscoleces of *Echinococcus granulosus in vitro*. *Plant Arch*. 2019;19:843-846.
14. **Mohammed ZN, Hussein ZA, Yousif JJ.** Scolicidal effect of ethanolic extract for *Cydonia oblonga* seeds in the viability of the protoscolices of the echinococcus granulosus parasite *in vitro*. *Indian J Public Health.* 2019;10(6).
15. **Jawad MJ, Rasool MI, Owadh HK, et al.** Anti-oxidant effect of infliximab and DMF during ischemia reperfusion induce liver injury in male rat. *J Pharm Negat Results*. 2022;13(3):685-689.
16. **Uwakwe OS, Abalinda GM, Pius T, et al.** Prevalence and antimicrobial susceptibility of urinary tract pathogens among female students visiting Kampala International University Teaching Hospital in Bushenyi, Uganda. *Elite J.*
17. **Hoffmann KF, Wynn TA, Dunne DW.** Cytokine-mediated host responses during schistosome infections; walking the fine line between immunological control and immunopathology.
18. **Mateen S, Zafar A, Moin S, et al.** Understanding the role of cytokines in the pathogenesis of rheumatoid arthritis. *Clinica Chimica Acta.* 2016;455:161-171.
19. **Joosten LA, Abdollahi-Roodsaz S, Dinarello CA, et al.** Toll-like receptors and chronic inflammation in rheumatic diseases: new developments. *Nat Rev Rheumatol.* 2016;12(6):344-357.
20. **Huang QQ, Pope RM**. The role of toll-like receptors in rheumatoid arthritis. *Curr Rheumatol Rep*. 2009;11(5):357-364.