DOI: https://doi.org/10.31351/vol33iss1pp163-171

Effect of Docetaxel on Matrix Metalloproteinase 1 Expression in Freund's Adjuvant Induced Arthritis

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Abstract

Rheumatoid arthritis is an autoimmune disorder that affects the synovial joints; and it leads to inflammatory changes that can occur in the synovial tissue, cartilage, bone, and, less frequently, extra-articular structures. Docetaxel, is an anti-neoplastic drug. Methotrexate is a treatment for early-stage rheumatoid arthritis but have 30% failure in treatment within the first year due to resistance or side effects. Matrix metalloproteinase 1 is the proteinase enzyme that is primarily in charge of the irreversible degradation of cartilage, bone, and tendons in the joints, which are induced by cytokines. This study aims to assess the efficacy of docetaxel in treating arthritis by examining the expression of matrix metalloproteinase 1 and comparing it to the effects of methotrexate and a combination of methotrexate and docetaxel in Freund's induced arthritis. Additionally, the study measured levels of Anti-Cyclical Citrullinated Peptide Antibody and Tumour Necrosis Factor-Alpha in both serum and tissue homogenate, respectively, as well as changes in ankle joint circumference.

To induce arthritis, complete Freund's adjuvant is injected subcutaneously into rats. From the 40 male Wister rats, five groups of eight animals were formed by random selection. The first (control) group was composed of non-diseased rats. The second to fifth group were induced by complete Freund's adjuvant and 0.5ml of normal saline that was intraperitoneally-injected to both the control and induction groups. The third group was administered 1mg/kg of Docetaxel on alternate day based on a preliminary experiment. The fourth group was given 1 mg/kg/week of Methotrexate intraperitoneally. The fifth group was received a half dose of Methotrexate and Docetaxel simultaneously. Serum level of Anti Cyclical citrullinated Peptide Antibody, tissue homogenate level of tumor necrosis factor-alpha, knee joint circumference and Immunohistochemical of Matrix metalloproteinase 1 measurements were applied. A significant decrease in serum level of Anti-Cyclic citrullinated Peptide Antibody, tissue homogenate level of tumor necrosis factor-alpha and Knee joint circumference in the Docetaxel group. A significant lowering in the expression of Matrix metalloproteinase 1 immunoreactivities in Docetaxel and Methotrexate groups with almost absent spots of positive reaction with the matrix of synovial tissue, hyaline cartilage & articular surface**.** This study showed that Docetaxel has anti-arthritic effect through their significant lowering expression of Matrix metalloproteinase 1 immunoreactivities.

Keyword: Rheumatoid arthritis, Docetaxel, Matrix metalloproteinase 1, Methotrexate, Tumor necrosis factor-alpha, citrulline.

تأثير الدوسيتاكسيل على وجود ماتريكس ميتالوبروتنيز النوع ١ في التهاب المفاصل الناجم عن مساعدة فرويند

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الخلاصة

التهاب المفاصل الروماتويدي هو مرض مناعة الذاتية يؤثر على المفاصل الزليليّة ويؤدي إلى حدوث تغيرات التهابية في النسيج الزليلي، والغضاريف، والعظام، وفي الأحيان اقل في الاجزاء خارج المفصل. الدوستاكسيل دواء مضاد للأورام. الميثوتريكسات هو دواء لمرحلة مبكرة من التهاب المفاصل الروماتويدي ولكن لديه فشل علاجي بنسبة ٣٠٪ خلال السنة الأولى بسبب المقاومة أو الآثار الجانبية. ماتريكس ميتالوبروتنيز النوع ١ هي بروتيناز المسؤول بشكل أساسي عن التدهور غير الانعكاسي للغضاريف والعظام والأوتار في المفاصل، والتي تحدث بسبب السيتوكينات. تهدف هذه الدراسة إلى تقييم التأثير المضاد للالتهاب لدوسيتاكسيل على تعبير مادة ماتريكس ميتالوبروتنيز النوع ١ ومقارنته بالميثوتريكسات والميثوتريكسات مع الدوسيتاكسيل في التهاب المفاصل الناجم عن فرويند ومستوى المصل الخاص بالجسم المضاد للببتيد الحلقي السيترولي المضاد، ومستوى تجانس الأنسجة لعامل نخر الورم. الطرق: للحث على المرض، يتم حقن مساعد فرويند الكامل تحت الجلد في الجرذان. من ٤٠ ذكور جرذان الوستر، تم تشكيل خمس مجموعات من ثمانية جرذان. تتكون المجموعة الضابطة من الجرذان غير مريضة. تم تحفيز كل من المجموعة الثانية الى المجموعة الخامسة بواسطة مساعد فرويند الكامل وتم إعطاء ٠,٥ مل من المحلول الملحي العادي داخل الصفاق لكل من مجموعتي الضابطة والمستحثة. يتم إعطاء المجموعة الثالثة ١ مجم / كجم / كل يومين دوستاكسيل بناءً على تجربة أولية. المجموعة الرابعة وتعطى ١ مجم / كجم / أسبوع من الميثوتريكسات داخل الصفاق. المجموعة الخامسة تتلقى نصف جرعة من ميثوتريكسات ودوسيتاكسيل في وقت واحد.

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Received: 2/2 /2023
Accepted: 4/5 /2023

تم تطبيق قياس مستوى المصل الخاص بالجسم المضاد للببتيد الحلقي السيترولي المضاد، ومستوى تجانس الأنسجة لعامل نخر الورم ومحيط مفصل الركبة والمناعية الكيميائية ماتريكس ميتالوبروتنيز النوع ١. النتائج: انخفض معنوي في مستوى المصل الخاص بالجسم المضاد للببتيد الحلقي السيترولي المضاد، ومستوى تجانس الأنسجة لعامل نخر الورم ومحيط مفصل الركبة في مجموعة دوسيتاكسيل
. انخفاض كبير في التعبير عن النشاط المناعي لماتريكس ميتالوبروتنيز النوع ١ في كل من مجموعتي الدوسيتاكسيل والميثوتريكسات مع شبه غياب لبقع من التفاعل الإيجابي مع مصفوفة النسيج الزليلي والغضروف الهيالييني والسطح المفصلي. الخلاصة: أوضحت هذه الدراسة أن مادة الدوسيتاكسيل قد يكون لها تأثير مضاد لالتهاب المفاصل من خلال تعبيرها الخفضي المعنوي عن نشاط بروتين ماتريكس ميتالوبروتيناز ١ المناعي.

الكلمات المفتاحية: التهاب المفاصل الروماتيودي, الدوسيتاكسيل, ماتريكس ميتالوبروتيناز ١, الميثوتريكسات, عامل نخر الورم, سترولين

Introduction

Rheumatoid arthritis (RA) is a prevalent immune-mediated inflammatory disease that primarily affects synovial joints; where, the joint immune cell infiltration is the hallmark of such disease [(1)]; since, RA is characterized by inflammatory changes in synovial tissue, cartilage, and bone, as well as less frequent extra-articular structures [(2)]. Annual incidence and prevalence rates for RA are 3 cases per 10,000 people and 1%, respectively, on a global scale [(3)]. In Iraq, it was observed an increase in RA incidence from 1.60 % in 2001 to 3.02% in 2011 [(4)]. The dysregulated synthesis of citrullinated proteins in RA joints is referred to as RA citrulline which is one of the crucial steps in the autoimmune response [(5)]. Pathogenesis involves TNF-α, IL-1β, IL-21, and IL-17 [(6)]; and the majority of immune cells in the RA-inflamed joints are neutrophils, and their capacity to form neutrophil extracellular traps (NETs) contributes to RA pathogenesis by generating autoantibodies and activating Fibroblast-like synoviocytes (FLS) [(7)]. Moreover, the activated neutrophils release immunological mediators like IL-1β, IL-6, IL-12, TGF-β, and TNF-α that can cause acute and chronic inflammation [(8)]. Furthermore, cytokines are endogenous peptides with high potency and pleiotropy [(9)]. Additionally, macrophages, these cells play a crucial role in producing cytokines [(10)].

Matrix metalloproteinases (MMPs) are proteinases in charge of the irreversible degradation of cartilage, bone, and tendons in the joints, which are induced by cytokines [(11)]. Furthermore, the TNF-alpha and IL-1beta control the expression of the MMP gene *via* signal transduction pathways, such as those controlled by mitogen-activated protein kinases (MAPKs) [(12)]. Moreover, the inflamed synovium growth factors promote the synovial fibroblast growth, along with immune cells and macrophages, forming a pannus; and due to its aggressive and invasive nature, pannus has been compared to localized cancer [(11)].

Fibroblast like synoviocytes (FLS) release inflammatory cytokines and MMP, which play a role in rheumatoid arthritis pathophysiology [(13)]. Moreover, the MMP-1 and MMP-3 are directly associated with the bone degradation of RA [(14)]; and the MMP-1 is crucial for several physiological functions, including tissue growth and development [(11)].

A semi-synthetic version of the taxoid anti-cancer drug Paclitaxel (PTX) is called Docetaxel (DTX) [(15)], which has four times the antiangiogenic activity of Paclitaxel [(16)]; and according to earlier research, PTX can produce lower levels of VEGF, TNF-α, and IL-1β than the induction group [(17)]; furthermore, PTX is a reversible peptidyl arginine deiminase (PAD) inhibitor beside other drugs (e.g., minocycline, and streptomycin) [(18)]. Additionally, researchers found that patients who have been previously-diagnosed with RA and are undergoing cancer treatment may experience anti-arthritic effects from either docetaxel-cisplatin or docetaxel-carboplatin [(19,20)].

Methotrexate (MTX), is a conventional synthetic disease-modifying antirheumatic medications (csDMARDs) which is used to treat early-stage RA[(21)]; but about 30% of those patients on MTX have been stopped within the first year of the treatment because it is ineffective or has side effects [(22)].

Aim of the study

The present study aims to assess the antiarthritic efficacy of docetaxel (DTX) by evaluating its impact on the expression of matrix metalloproteinase 1 (MMP-1), and comparing it with methotrexate (MTX), as well as a combination of MTX and DTX, in the context of Freund's induced arthritis. The study will also investigate the antiarthritic effect of DTX through the measurement of serum levels of Anti-Cyclical Citrullinated Peptide Antibody (ACPA) and tissue homogenate levels of tumour necrosis factor-alpha (TNF-α).

Material and Methods

Animals

A total of forty (40) male Wister rats aged 12 to 14 weeks weighing 200 to 250g were obtained from the University of Tikrit's College of Veterinary Medicine; and for the animals' comfort, a temperature of 255°C was maintained, and an artificial light unit was used to create a light/dark cycle; and, the animals have unrestricted access to food and water in the animal house at the College of Pharmacy, Al-Mustansiriyah University; and this study begun after receiving approval from the ethics committee of the College of Pharmacy; and, it was conducted between November 2021 and May 2022. The animals were randomly-assigned into five (5) groups of eight (8) animals each; where, the **first** group served as the control/healthy rats. **The second to fifth** groups of rats were induced with Complete Freund's adjuvant (CFA) by subcutaneous-injection (Sc) at the base of the tail and hind paws [(23)]. Both the control and induction groups were

intraperitoneally (IP)-injected with 0.5ml of normal saline (NaCl).

Moreover, the **third** group of rats were treated with 1mg/kg of DTX every other day, based on our preliminary experiment; and the **fourth** group IP injected with 1mg/kg/week of MTX [24]; and the **fifth** group was the combination group, which received half doses of both MTX and DTX.

On days 0 and 10 of induction, rats in the DTX, MTX, and DTX+MTX groups were given 1.2 and 0.4 ml of CFA at their tail bases; since DTX and MTX were administered to these animals as usual until the day 33. Then, blood was withdrawn from the heart of each rat, and collected in gel tubes, and centrifuged at a rate of 3000 rotations/15 minutes to obtain serum. Additionally, the articular tissue was collected, weighed, chopped into small pieces, and homogenized by using a specific volume of phosphate buffer saline (PBS) (usually 10mg tissue to 100µl PBS); since, the homogenization was performed for about 20minutes at 3000 rpm, and the supernatant was carefully-collected.

Furthermore, the activated T cells populate the inflamed joints of AIA rats were determined in rats, which induced by Sc injection of the CFA either at the base of the tail or between the hind paws.

Ankle joint circumference

The processes of inflammation and edema were also examined by measuring joint circumference using the following equation [4,25]:

$$Circumference = 2\pi\sqrt{\frac{a^2 + b^2}{2}}$$

Where: circumference: joint circumference, Л: 3.14, a: Mediolateral diameter of joint, b: Anteroposterior diameter of joint Stretching the rat joint was used to measure each diameter, and then the Micrometer was utilized to make the final judgments.

Serum level of Anti Cyclical citrullinated Peptide Antibody, and tissue homogenate level of tumour necrosis factor-alpha measurement

Serum level of Anti Cyclical citrullinated Peptide Antibody (ACPA) and the articular tissue homogenate level of TNF-α, were determined with available ELISA kits (My BioSource's, USA) as the manufacturer's instruction.

Immunohistochemical staining of the matrix metalloproteinase 1 (MMP1)

Immunohistochemistry allows the visualization of specific cellular components in tissue[26]. This staining method followed the manufacturer's brochure and used histopathological blocks[27]. the slides were photographed using a light microscope and digital camera. The IHC staining score method, which combines stain intensity and the fraction of positive cells, is used to evaluate anti-MMP1 antibodies.

1. Intensity from 1(Weak), 2 (Moderate) to 3 (Sever).
2. Immunoreactive cells grade 0 for less than 10%, grade 1 (+) for 10-30%, grade 2 (++) for 31-50%, grade 3 (+++) for 51-70 % and grade 4 (++++) for more than 71 of reactive cells[28].

Statistical analysis

The data were presented in the form of means ± standard deviation (M ± SD). Data were analyzed with SPSS-20.0. To analyze various means, the analysis of variance (ANOVA) and the post-hoc Tukey test were used. P-values equal or less than 0.05 are statistically-significant. Moreover, the Kruskal-Wallis and Mann-Whitney tests were used for non-parametric statistical analysis of nominal data[29].

Results

Experimental assessment of Rheumatoid Arthritis

In the induction group of rats, there was a statistically-significant *($p \leq 0.05$)* increase in the KJC compared to such conference in the control group on the final day of the study; moreover, in the DTX-treated rats/ Group 3, there was a significant decrease *($p \leq 0.05$)* in KJC compared to the induction/ Group 2 rats; while, in the MTX and DTX+MTX groups (Groups 4 and 5), respectively there was only a marginal decrease relative to the induction/Group 2; since, these findings are presented in Table 1.

Table 1. Knee joint circumference.

Groups	Number of the lab. Animals	Knee joint circumference (mM)
1/Control	8	20.513 ± 0.564 a
2/Induction by CFA	8	24.933 ± 2.765 b
3/DTX	8	21.577 ± 1.367 a c
4/MTX	8	22.806 ± 0.850 b c
5/DTX+MTX	8	23.784 ± 1.399 b c

Data are mentioned as means± SD

CFA: Complete Freund's adjuvant, DTX: docetaxel, MTX: methotrexate, mM: millimeter

Different lowercase letters indicate significant differences between groups *($P \leq 0.05$)*

Serum level of Anti Cyclical citrullinated Peptide Antibody (ACPA) & Tissue homogenate level of tumor necrosis factor-alpha (TNF-α)

In the induction/Group 2 rats, there was a significant higher level of ACPA and TNF-α in serum and tissue homogenates, respectively compared to the control/Group 1 *($p \leq 0.05$)*. However, in the treatment groups (DTX/Group 3, MTX/Group 4, and DTX+MTX/Group 5), there was a significant decrease *($p \leq 0.05$)* in ACPA and TNF-α levels compared to the induction/Group 2; since, the statistical results are presented in Figure 1 and Figure 2, respectively.

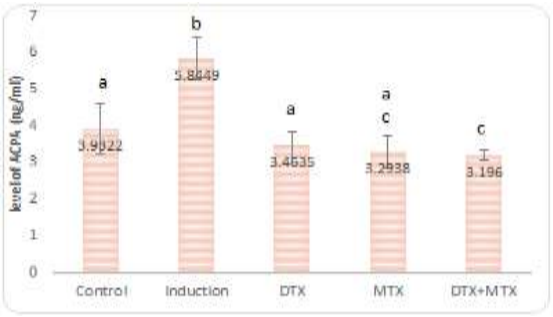


Figure 1. Anti Cyclical citrullinated Peptide Antibody (ACPA) among studied groups

Data are demonstrated as means± SD. Induction Group/Complete Freund's adjuvant (CFA), DTX= docetaxel, MTX= methotrexate.Different lowercase letters indicate significant differences between groups *($p \leq 0.05$)*

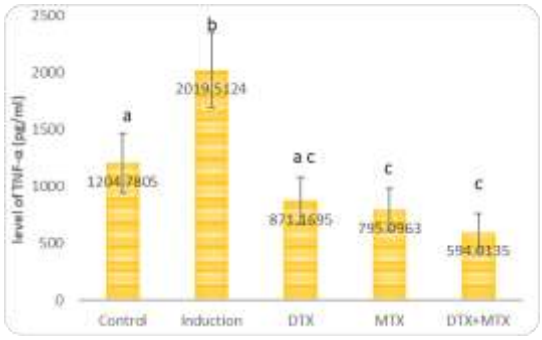
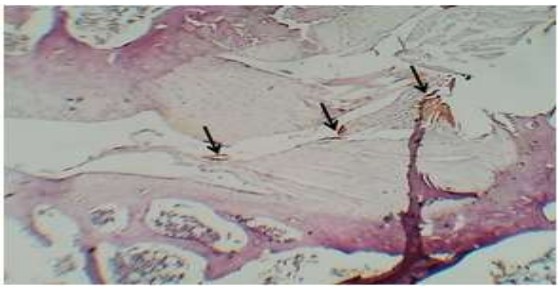


Figure 2. Tumor necrosis factor alpha (TNF-α) among studied groups

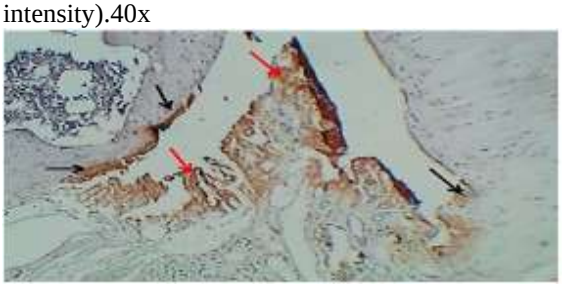
Data are mentioned as means± SD .Induction Group/Complete Freund's adjuvant (CFA), DTX= docetaxel, MTX= methotrexate .Different lowercase letters indicate significant differences between groups *($p \leq 0.05$)*

Immunohistochemical examination for matrix metalloproteinase1 (MMP1) biomarkers

Semi quantitative scoring (Ordinal scale of scores) of combined stain intensity and percent of cells positive approach for each group is displayed in (figure 3).

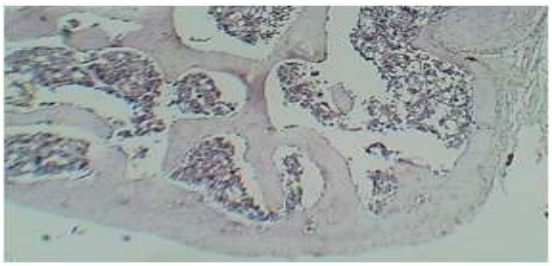


A grade 1 (3 spots of positive reaction with severe intensity).40x

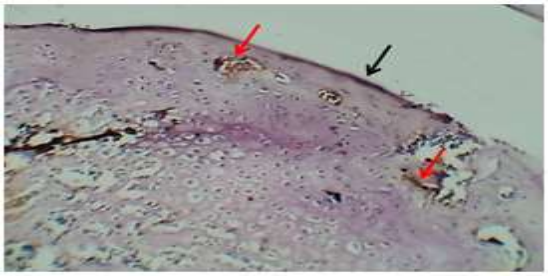


B grade 3 (spots of positive reaction on the articular surface (black arrows) and within synovial tissue (Red arrows) with severe intensity).40x

C grade 0 (no spots of positive reaction whine joint)40x



D grade 0 (no spots of positive reaction within joint).40x



E: grade 1 (Few spots of positive reaction on the articular surface (black arrows) and within matrix (red arrows) with moderate intensity).40x

Figure 3. Immunohistochemistry staining of mmp1 expressed in knee joint of different groups. A: Control group, B: Induction group/Complete Freund's Adjuvants (CFA), C: DTX group, D: MTX group, E: DTX+MTX group.

Immunohistochemical scoring among studied groups

The immunohistochemical scoring was obtained, and shown in (table 2); where, in terms of stain intensity score, the mean rank of control group (27.50) showed a significant difference *(p≤0.05)* compared with induction/Group 2/CFA (34.69). Furthermore, the mean rank of DTX/Group 3 is (9.50), MTX/Group 4 is (11.00) and in DTX+MTX is (19.81) and showed a significant decrease *(p≤0.05)* each compared to such scoring in the induction/Group 2/CFA rats.

Concerning the term of the immunoreactive cells grade, the mean rank in the induction/Group 2/CFA was significantly-increased *(p≤0.05)* which is (36.50) compared to such cell grade in control Group 1, which is (18.00). Additionally, the mean rank of -DTX/Group 3 is (14.00), -MTX/Group 4 is (14.00) and -DTX+MTX is (20.00); where, each is significantly-decreased *(p≤0.05)* compared to that in the induction by CFA/Group 2 rats. As indicated in (table 2) and (figure 4).

Table 2. Mean rank of Stain intensity score and Immunoreactive cells grade of mmp1 expressed in knee joint of different groups.

Group	Stain intensity score		Immunoreactive cells grade	
1/Control	**Sever**	27.50 a	+	18.00 a
2/Induction	**Sever**	34.69 b	+++	36.50 b
3/DTX	**0**	9.50 c	**0**	14.00 a
4/MTX	**0**	11.00 c	**0**	14.00 a
5/DTX+MTX	**Moderate**	19.81 a	+	20.00 a

Data represent mean of rank
Induction group/Complete Freund's Adjuvants (CFA), DTX: docetaxel, MTX: methotrexate
Different lowercase, uppercase letters indicate significant differences between groups *(p ≤ 0.05)*.

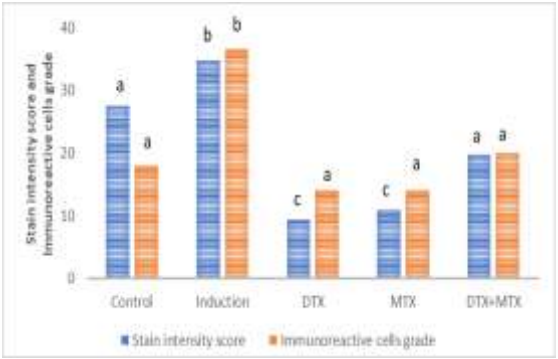


Figure 4. Mean rank of Stain intensity score and Immunoreactive cells grade of MMP1 expressed in the knee joint of different groups.

Data represent mean of rank Induction group/Complete Freund's Adjuvants (CFA), DTX: docetaxel, MTX: methotrexate MMP1: matrix metalloprotease-1 . Different lowercase letters indicate significant differences between the groups *(p ≤ 0.05)*

Discussion

Experimental assessment of Rheumatoid Arthritis

There was a high KJC in rats of Group 2 that was induced by CFA in the current study; since, such results are align with those of others; where, in the induction group there was more edema and a larger ankle diameter (cm) compared to that of the control group [30].

Furthermore, in terms of KJC reduction, in the Group 3/DTX-treated rats, there was a lowest value, followed by MTX/Group 4, and then the combination (DTX+MTX)/Group 5, as illustrated in Table 1; since, these results are consistent with those of El-Tedawy and colleagues (2020), who reported that MTX reduced knee joint swelling circumferences [31].

Serum level of Anti Cyclical citrullinated Peptide Antibody (ACPA) & Tissue homogenate level of tumor necrosis factor-alpha (TNF-α)

In this study, the serum level of ACPA is significantly-increased in the induction by CFA/Group 2 compared such serum level in the control/Group 1 (*P≤0.05*) (Figure 1); and, results of this study are consistent with those of other researchers who found that the mean serum ACPA level is significantly-increased compared with the control group[32]. Moreover, Grassi and his colleagues (2022) found that after the injection of the CFA, the was also elevated raised[33].

In the present study, there was a significant increase in the serum level of ACPA in the induction by CFA/Group 2 rats compared to such serum level in the control/Group 1 (P ≤0.05); and such results are consistent with the findings of a previous study [32]. Similarly, Grassi et al. (2022) reported a significant increase in ACPA levels after the induction of CFA [33].

Furthermore in the current study, both DTX-(Group 3) and MTX-treated/(Group 4) rats demonstrated a significant ability to decrease the serum level of ACPA compared to such serum level in the induction by CFA/Group 2 rats, and there was no-significant difference between the two treatment groups (Figure 1); and the results of this study are inconsistent with a previous stud, which showed that there was a significant reduction in ACPA levels in the MTX-treated group compared to corresponding level in the induction group [34].

Interestingly, in the combination group/DTX+MTX (Group 5), there was a reduction in the serum level of ACPA below to that of the control/Group 1 (Figure 1), this may be attributed to the presence normal level of ACPA in healthy rats, as a human anti-CCP level below 20 units is considered normal and healthy, while a level above 20 units is indicative of RA suspicion [35].

Furthermore, the TNF-α/a well-known inflammatory cytokine involved in the RA, was assessed in this study; where, in the induction by CFA/Group 2 rats, there was a significant increase in the TNF-α tissue homogenate level compared to that level in the control/Group 1 (Figure 2); where, such result is consistent with previous studies indicating an increase in inflammatory cytokine levels in RA [36,37].

Moreover, in the DTX-treated/Group 3 rats, the TNF-α level significantly-decreased and reached the control/Group 1 rats (Figure 2) and such results are consistent with those of others showing that DTX has a more prominent impact on lowering IL-1β and TNF-α levels in other disease (advanced breast cancer) patients undergoing chemotherapy [38]. Additionally, Sheng et al. (2020) also found significantly-greater TNF-α level in the induction group compared to such level in control group; furthermore, researchers reported that, in treatment groups (Paclitaxel (PTX)/other taxane drug, and MTX) there were a reduction in the TNF-α levels compared to the induction group [39].

Moreover, in the MTX-treated group of the current study showed a decrease in TNF-α level, (Figure 2), and such results are similar to those of other researchers which indicate that MTX suppressed the TNF-α and limited its activity *in vitro* [40].

Concerning the combination (DTX+MTX)/Group 5 rats, there was a greater decrease in TNF-α level compared to each of DTX or MTX alone (Figure 2); and suggest an additive effect between DTX and MTX on TNF-α level; and such result is consistent with those of Sun et al (2020) who also observed a decrease in TNF-α levels below normal levels in their TNF-α treatment group [41].

Furthermore, the increase in TNF-α level in this study could be explained by the stimulation of TNF-α generation from macrophages by immune complexes composed of solid-phase citrullinated fibrinogen and ACPA [42]; moreover, researchers mentioned that the TNF-α also controls the expression of the MMP gene through signal transduction pathways, such as those controlled by mitogen-activated protein kinases (MAPKs) [12].

Immunohistochemical expression of matrix metalloproteinases 1 (MMP1)

The scoring of the induction group by CFA/Group 2 showed that there is a significant increase in MMP1 expression in this group compared to that expression in control/Group 1 rats; since, the presence of numerous spots of positive reaction with severe intensity in the matrix of synovial tissue, hyaline cartilage, and articular surface indicates that MMP1 may be involved in the degradation of these structures in the Induction group (Table 2) and (figure 3).

A previous study observed that in the control monkeys there were low levels of MMP-1 immunoreactivity in the cells lining the articular cartilage and synovial tissues, suggesting normal collagen degradation; however, in the induction animals exhibited higher levels of MMP-1 immunoreactivity in these same areas [43]; additionally, a previous study also found an increase in MMP1 expression in RA synovia compared to non-RA synovia [44]; and the overexpression of MMP1 may contribute to the pathogenesis of RA by promoting the destruction of articular tissues.

Furthermore, in the current study, both DTX-treated/Group 3 and in MTX-treated/Group 4 rats, there was a decrease in MMP1 immunoreactivity in the articular tissue, with grade zero scores indicating a near absence of MMP1 expression. There are no study concerning the effects of DTX on MMP1 expression; but previous studies suggested that PTX and MTX drugs each had anti-inflammatory effects that contributed to the reduction in MMP1 expression; furthermore, another study and by using northern blotting, the other taxane drug (PTX) is a powerful anti-inflammatory drug by inhibiting MMP-1 and MMP-3 production at the Activator protein 1 (AP-1) site [45]. Also, another study using ELISA serum found that PTX most probably contributed to the MMP-1 expression in human Tenon fibroblast (HTFs) being downregulated [46]. Moreover, researchers mentioned that MTX significantly-lower the serum level of MMP1 compared to such serum level in the induction group [47]. Additionally, another previous study found that the results of Western blotting showed that MTX significantly-reduced the levels of VEGF and MMP-1, 2 & 9 in serum[48].

In contrast, in the combination group (DTX+MTX)-treated rats of this study, there was a few spots of positive reaction with moderate intensity and a grade 1 score, as shown in (Table 2 and figure 3), and this indicating a partial reduction in MMP1 expression; and no results are performed by others; thus, we do not have the chance to compare the results of the present study concerning the above respects with others.

Overall, these findings suggest that DTX and MTX may have potential as treatments for RA due to their ability to reduce MMP1 expression, although further research is needed to confirm these results.

Conclusion

According to the results of this study, docetaxel demonstrated promising potential as an anti-RA drug, as it significantly reduced the

expression of MMP1 and lowered ACPA, TNF-α, and knee joint circumference levels.

Acknowledgments

The authors thank the college of pharmacy, Mustansiriyah University https: // www. uomustansiriyah.edu.iq for approving and supporting this study, which is part of the dissertation of a master's student.

Conflicts of Interest

The authors assert that there is no bias, competition, or conflict of interest present, as stated.

Funding

This study did not receive funding from any government, business, or nonprofit organization.

Ethics Statements

The research, conducted between November 2021 and May 2022, received approval from the Ethics Committee at the College of Pharmacy, Mustansiriyah University.

Author Contribution

O.M.A., G.A.J., and S.Y.J. were involved in designing the research. O.M.A. conducted the experiments and collected samples, while O.M.A. and G.A.J. analyzed the data. O.M.A. authored the manuscript, which was reviewed by G.A.J. and S.Y.J., who also provided supervision throughout the project. All authors reviewed and approved the final manuscript.

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