

Cuscuta Chinensis potentiate the effect of methotrexate in Rheumatoid Arthritis Induced Rats.

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Abstract:

Background

Rheumatoid arthritis (RA) is a chronic destructive inflammatory disease related to a breakdown in immune tolerance. This disease is characterized by joint inflammation, swelling, and in severe cases deformation may occur. Cuscuta

Chinensis (C. Chinensis) is a parasitic plant, grow around other plants to absorb nutrient and water from them. C. Chinensis has a wide range of chemicals that produce a wide range of pharmacological activates. Because of its anti-oxidant and anti-inflammatory effect, it was considered as a good candidate to assess its role in RA.

Methods:

Rheumatoid arthritis was induced by injection of Complete Freund's Adjuvant inside the foot-pad of male albino rats. The animals were grouped in four groups as follows group 1 considered as a normal control group, group 2 consider as positive control arthritis, group 3 treated with methotrexate (MTX), group 4 treated with MTX and C. Chinensis extract. On day 14 of immunization, treatments began and last for 21 days, at the end of the experiment all animals were sacrificed and serum was collected. The serum markers that had been evaluated were MMP3, VEGF, and SOD. Throughout the experiment time the body weight was evaluated.

Results

The combination significantly ($P\text{-value} \leq 0.05$) improves objective parameter of RA which was the body weight. Also significantly decrease ($P\text{-value} \leq 0.05$) the serum level of MMP3, VEGF, and considerably increase serum SOD.

Conclusion:

The combination has a significant beneficial role in suppression of destructive enzyme (MMP3), angiogenic stimulators (VEGF), and increase serum SOD enzyme.

Key words: Rheumatoid arthritis, Cuscuta Chinensis, MMP3, VEGF, SOD.

نبات الحامول يزيد من فائدة الميثوتركسيت في علاج التهاب المفاصل الروماتيزي المستحث في الجرذان

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الخلاصة:**الخلفية**

التهاب المفاصل الروماتويدي هو مرض التهابي مزمن ومنمّر يحدث بسبب كسر التحمل المناعي. يتميز هذا المرض بالتهاب المفاصل والتورم ، وفي الحالات الشديدة قد يحدث تشوه. الحامل هو نبات طفيلي ينمو حول النباتات الأخرى لامتصاص المواد الغذائية والمياه منها. من المركبات ذات القيمة الدوائية لديها مجموعة واسعة من المواد الكيميائية التي تنتج مجموعة واسعة من التأثيرات الدوائية. نظرًا لتأثيرها المضاد للأوكسدة والمضاد للالتهابات ، فقد تم عدها كمرشح جيد لتقييم أثرها في التهاب المفاصل الروماتويدي.

المواد وطريقة العمل

تم تحفيز التهاب المفاصل الروماتويدي عن طريق حقن مادة Complete Freund's Adjuvant داخل وسادة القدم للجرذان البيضاء. تم تقسيم الحيوانات الى أربع مجموعات على النحو التالي المجموعة ١ تعتبر مجموعة تحكم عادية ، المجموعة ٢ تعتبر التهاب المفاصل الضابطة الإيجابية ، المجموعة ٣ تعامل مع ميثوتريكسات (MTX) ، المجموعة ٤ تعالج بمستخلص MTX و C. Chinensis. في اليوم الرابع عشر من التحصين ، بدأت العلاجات واستمرت لمدة ٢١ يومًا ، وفي نهاية التجربة تم التضحية بجميع الحيوانات وجمع المصل. كانت علامات المصل التي تم تقييمها هي MMP3 و VEGF و SOD. طوال فترة التجربة تم تقييم وزن الجسم للجرذان.

النتائج

يعمل العلاج المركب بشكل ملحوظ (قيمة $P \leq 0.05$) على تحسين الوضع العام لمرضى الـ RA خصوصاً وزن الجسم. ينقص أيضاً بشكل ملحوظ (قيمة $P \leq 0.05$) مستوى مصل MMP3 ، VEGF ، ويزيد بشكل ملحوظ SOD المصل.

الخلاصة

تؤدي التركيبة دوراً كبيراً في كبت الإنزيم المدمر (MMP3) ، والمنشطات الوعائية (VEGF) ، وزيادة إنزيم SOD في الدم.

الكلمات المفتاحية: التهاب المفاصل الروماتيزمي. ميثوتريكسيت. SOD. VEGF. MMP3.

Introduction

Rheumatoid Arthritis (RA) is a chronic inflammatory disease from auto-immune origin. It characterized by swelling of joints and difficulty in movement started at the small joint then may spread to the large one in symmetrical pattern. RA also consider as distractive disease that may lead to bone erosion and joint deformity as an articular complication and multiple organ dysfunction as extra-articular manifestations [1].

Rheumatoid arthritis tends to affect people at any age, but mainly between 20 and 40 years. It affects about 1% of the population, about 20 per 50,000 people. RA occurred in females more than males in a 3:2 ratio [2]. RA incidence in Asian and European are more than the African nation, this was explained by Matthew Traylor *et al.* who found that genetic risk factors in Asian and European ancestry are more than African peoples [3]. In Iraq, RA may affect 1% of the population [4] and this percent also may vary in different governments, according to Ali Mohammed researches who document that RA affects 3.02% of the population in Babylon in 2011 [5].

The presented feature of RA patients may include pain, morning joint stiffness that lasts for more than one hour [2], low-grade fever, arthralgia due to local inflammation, weight loss, and fatigue that may be accompanied as nonspecific symptoms [6].

The initiation of RA may contribute to many risk factors some of these factors are genetics, others may be acquired due to a bad lifestyle.

Genetic factors can be considered as the main initiating factor. More than 100 Loci are associated with triggering, initiation, and progression of the disease [7]. The most important one are the loci for Major Histocompatibility (MHC) class2 gens mainly HLA DR01/ 04 which has an important role in recognition of auto-reactive peptides by T cells, and other rules in activation of stimulatory immune pathways for CD 40, CD 28, cytokines receptors, and chemokines [8]. Cigarette smoking considered as an environmental factor, it increases the risk of RA in male double than the non-smoker [9, 10]. Tobacco smoking induces Peptidylarginine deiminases (PADs) in the lung, and this may lead to increase citrullination [11, 12],

especially in genetically susceptible patients^[13]. Workers who are exposed to silica, industrial pollution, pesticide, and dealing with electronic row materials at high risk than others for the development of RA^[14-15]. The exposure to these noxious substances strongly associated with the induction of the immune system and increase the level of citrullination in the lung^[11]. Also, antibodies are formed from immune cells against bacterial or viral infections. Epstein-Barr virus (EBV) is a herpes virus that can infect both B cells and epithelial cells^[16], in some RA patient's high serum level of antibody against EBV was found, so infection with this virus consider as a risk factor^[17].

From another point of view, some bacterial infections like for example *Porphyromonas gingivalis* which cause either gingivitis or intraperitonitis, these bacteria increase the level of citrullination of some synovial protein like Fibronectin and Enolase.^[18] Also, infection with *Aggregatibacter actinomycetemcomitans* consider as a risk factor, because this pathogen will release leukotoxin A, which making a pore on neutrophil cell wall, leading to PAD leakage and start the process of citrullination^[20].

Female gender can be considered a risk factor for the development of the disease. This may be related to female ovarian hormones. Estradiol has a role in slowing down the signaling pathway of a pro-inflammatory cytokines^[21]. This may explain the high incidence in menopause females which could be related to the severe decline of this hormone^[22].

All *Cuscuta* species have a wide range of active components which have a lot of therapeutic uses^[23]. *Cuscuta Chinensis* Lam (*C. Chinensis*) belong to family *Convolvulaceae*, also it has other names like Chinese Dodder and Tu-Si-Zu^[24]. It's a parasitic plant, grow around other plants and depend on them to get the essential nutrients. *C. Chinensis* in general is yellow to green in color^[25].

Flavonoids (which have the main pharmacological properties), alkaloids, minerals, fatty acids, and essential oils are the main chemical component of *C. Chinensis*^[25].

It's widely distributed around the world mainly in Africa, Asia, Australia^[24], and grows abundantly in Iraq.

C. Chinensis extract has good oral bioavailability especially for flavonoids, also there is a piece of evidence showing that it may inhibit cytochrome P 450 enzymatic activity. It's a safe plant, with very low toxicity. In pregnancy, it has no mutagenic or teratogenic effect on pregnant rats and their offspring in a dose of up to 80 gm/kg^[24].

Methotrexate is a Disease-Modifying Anti-Rheumatic Drug (DMARD). These group of drugs are effective in the treatment of RA, but the exact mechanism of some of them is it still unknown like sulfasalazine. They mostly act as anti-inflammatory and immunomodulatory agents.

Aim of this study was to evaluate the additive effect of *Cuscuta Chinensis* on MTX treatment in RA induced in rats

Materials and Methods

The plant

Collection and identification

C. Chinensis was collected from the University of Babylon in May 2019, then successfully identified in the department of Biology/College of Science/University of Baghdad by Dr. Sukaina Abass Alawy. After that, the whole plant was dried at room temperature in shadow for 14 days.

Cuscuta Chinensis extraction and phytochemical screening

The whole dried plant was milled using mortar and pestle then finely grinded by electrical mills. The final powder then extracted via maturation process in water-methanol solvent in a ratio of 1:1. The final ratio of solvent to *C. Chinensis* dried powder was 10 ml: 1gm. The steps of extraction were explained in details by

Fadia *et al.* [26]. *C. Chinensis* fine extract powder was securely labeled and preserved in a sealed container at room temperature until be used later.

Phytochemical screening for *C. Chinensis* crude extract

A qualitative analysis for methanol watery extract of *C. Chinensis* for the presence of Alkaloids (Mayer's test), Flavonoids (Lead chloride test), Steroids (Liebermann test), and Polyphenolic compounds (Ferric chloride test) these tests mentioned by Sivapriya T *et al.* [27]

Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) is a technique used to separate and identify different compounds from a mixture or extract in a mobile phase system in various temperatures. This procedure is used to recognized hydrocarbons, glycosides, fatty acids, essential oils, and others [28].

Animals

Animals selection, housing, and feeding

Forty, non-previously treated male albino rats, aged (12 to 16) weeks, their weight was (220 ± 50 gm), obtained from the National Center for Drug Control and Research /Ministry of Health. These animals were housed in well-ventilated conditions and free access to food and water at $25^{\circ}\text{C} \pm 5^{\circ}$ in normal light/dark cycle in the animal house at College of Pharmacy/ Mustansiriyah University. Ethical committee from College of Pharmacy/ Mustansiriyah University had been given before starting this study which began in September 2019 and finished in January 2020.

Experimental design

Before initiating the main experiment, a preliminary study had been made to determine the effective dose of *C. Chinensis* crude extract against RF serum

level. The optimal dose was (500mg/kg/day).

Thirty rats were injected by (0.2, 0.1, and 0.05) ml on day (0, 5, and 10) respectively by Complete Freund's Adjuvant (CFA) obtained from Santa-Cruz Biotechnology, Inc. according to a previous preliminary study that had been performed to identify the optimal procedure for induction of RA. These animals were kept safe for 14 days after immunization, then the *C. Chinensis* crude extract and the stander therapy (Methotrexate) were used.

The dose of methotrexate used was (1 mg/kg/day) [29,30]. This dose was prepared by taking 1 ml (10 mg/ml) of methotrexate diluted by distal water (3 ml).

Animals grouping

After 14 days of immunization, all inducted animals had been grouped into three groups in addition to the normal animal group (group 1), group 2 control arthritis group, group 3: MTX, and group 4: combination group. Treatment lasts for 21 days, via gastric gavage for *C. Chinensis* crude extract and via intraperitoneal route (IP) for methotrexate.

Rat body weight.

Total body weight was evaluated every 7 days for all groups by using an electronic balance.

Serum markers

The rats were anesthetized by using ketamine (50mg/ kg) [31] IP. Then the blood was collected from the heart directly by using (10 CC syringe G23). The samples then centrifuged at (1000 rpm) for (15 min) in a gel tube to separate the serum [31]. Serum markers that were evaluated VEGF (Abcam Inc, USA), MMP₃ (Mybiosource, USA), and SOD (Mybiosource, USA).

Statistical analysis

All data analysis (which expressed as mean $\pm$ standard error of the mean) was performed using SPSS (V16.0), analysis of variance (ANOVA) test, in which the *P*-

value was less than 0.05 and the statistical significance has been considered

Results

Pharmacognostic study for *C. Chinensis* crude extract.

Preliminary extract screening

When the extraction process had been finalized, a preliminary screening for qualitative analysis for alkaloids, flavonoids, steroids, and polyphenolic compounds. The results were explained in the table (1).

Table (1): Results of preliminary extract screening.

Chemicals	Test for identification	Results
Alkaloids	Mayer's test	+
Flavonoids	Lead chloride test	++
Poly phenol	Ferric chloride test	+
Steroids	Liebermann test	+

++: Strong positive, strong color / high concentration.

+: Positive, presence.

Gas Chromatography-Mass

Spectrometry (GC-MS) analysis

The crude extract of *C. Chinensis* was also subjected to GC-MS, in order to identify other chemicals that may be found in the

extract and produce pharmacological actions. The most important identified chemicals were Thiirane, Carbamic acid, n-Hexadecanoic acid, Butylated hydroxytoluene, and Oleic acid. As shown in figure (1).

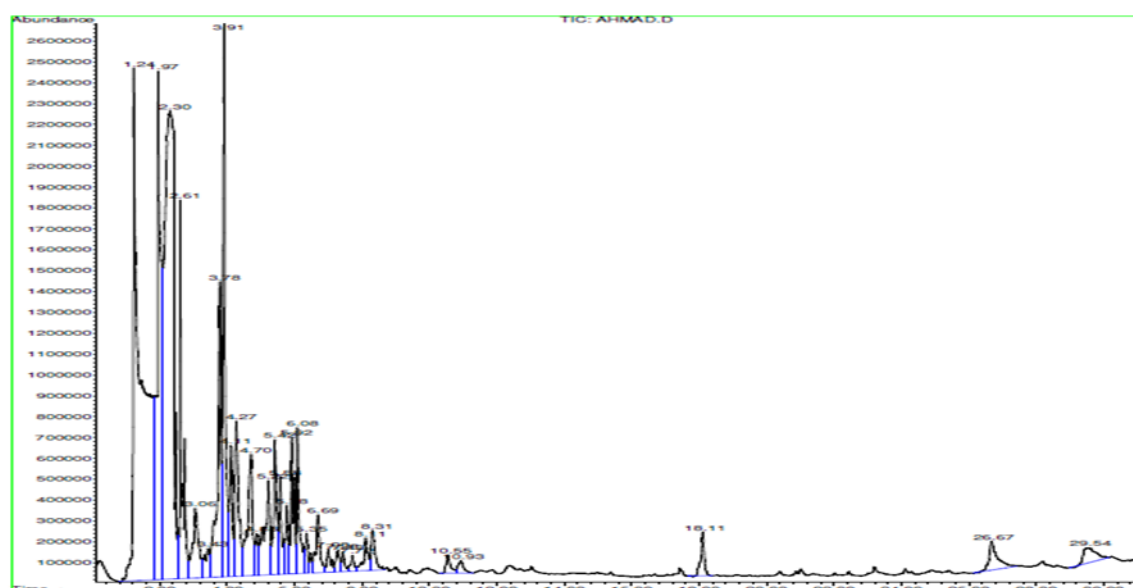


Figure (1): Gas Chromatography-Mass Spectrometry peaks for *C. Chinensis* methanol-watery extract.

Rat body weight:

Experimental groups (2-4) showed a decrease in body weight from the day (0 to 14), and the decrement in all immunized animals was significantly differed (P value

≤ 0.05) from group 1 on day 14. These data were illustrated in figure (2)

The maximum increase in body weight was shown in group 4. Also, this group was not significant (P value > 0.05) statically from group 1.

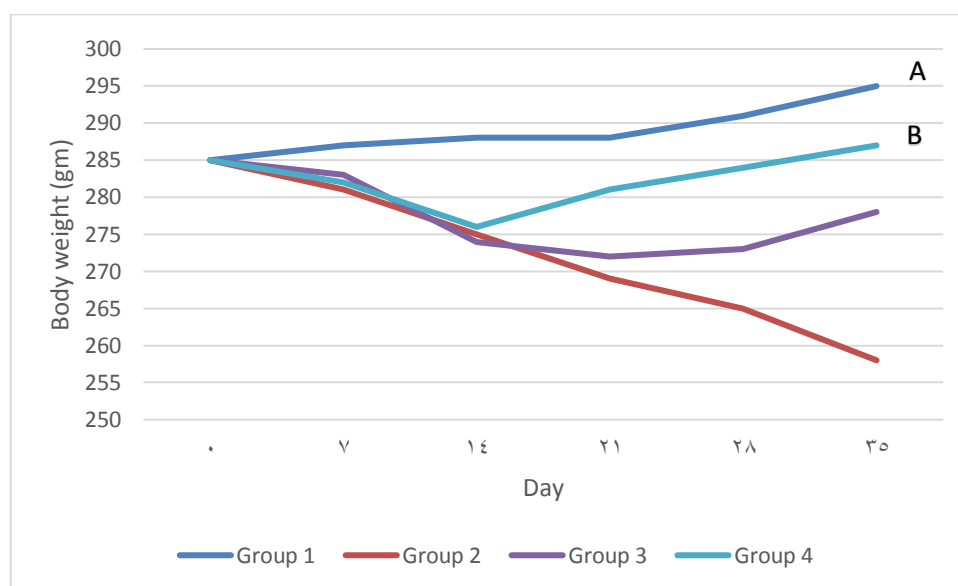


Figure (2): Changes in total body weight during experiment time.

Group 1: normal control group, Group 2: immunized group. Group 3: treated group with MTX. Group 4: group treated with a combination treatment of *C. Chinensis* crude extract and methotrexate. Letters with different capital letters is significantly differ. $P\text{-value} \leq 0.05$.

Serum Markers

Serum VEGF

The serum level of VEGF was significantly ($P\text{ value} \leq 0.05$) decrease in all treated groups (3,4 groups). Result from group 3 showed that MTX had an

inhibitory effect ($86.88 \pm 4.40\text{ pg/ml}$). When *C. Chinensis* added to MTX in group 5, the inhibitory effect decreases to reach ($78.57 \pm 4.74\text{ pg/ml}$), as shown figure (3).

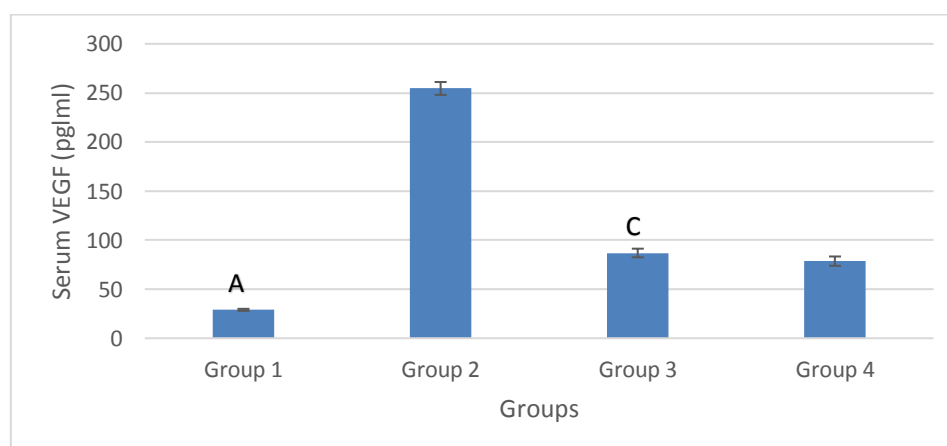


Figure (3): Changes in serum level of VEGF.

VEGF: Vascular Endothelial Growth Factor. Group 1: control (-ve) group, normal control. Group 2: control positive group. Group 3: Group treated with MTX. Group 4: group treated with a combination treatment of *C. Chinensis* crude extract and methotrexate. Data expressed as means $\pm$

SEM. Letters with different capital letters is significantly differ. $P\text{-value} \leq 0.05$.

Serum MMP₃

After 35 days of immunization, the serum level of MMP₃ was significantly upsurge ($P\text{ value} \leq 0.05$) in group 2 which was

(10.73 ± 0.28 ng/ml). *C. Chinensis* crude extract augment the inhibitory effect of MTX on serum MMP₃ significantly from

(6.70 ± 0.15 ng/ml) to (4.91 ± 0.19 ng/ml) (P value ≤ 0.05). Figure (4) show these findings.

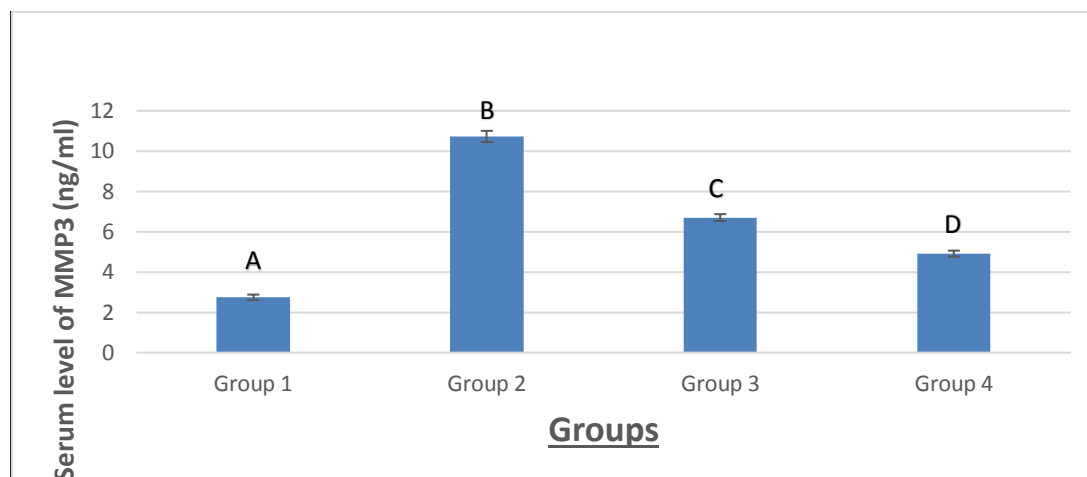


Figure (4): Changes in serum level of MMP₃.

MMP₃: Matrix Metalloproteinase 3. Group 1: control (-ve) group, normal control. Group 2: control positive group. Group 3: Group treated with MTX. Group 4: group treated with a combination treatment of *C. Chinensis* crude extract and methotrexate. Data expressed as means $\pm$ SEM. Letters with different capital letters is significantly differ. P -value ≤ 0.05 .

Serum SOD

C. Chinensis significantly (P value ≤ 0.05) palliate the oxidative stress of MTX treatment by enhancing the activity of

antioxidant enzymes (SOD) level from (60.72 ± 2.49 ng/ml) in group 3 to (92.72 ± 1.5 ng/ml) in group 4 (P value ≤ 0.05). As shown figure (5).

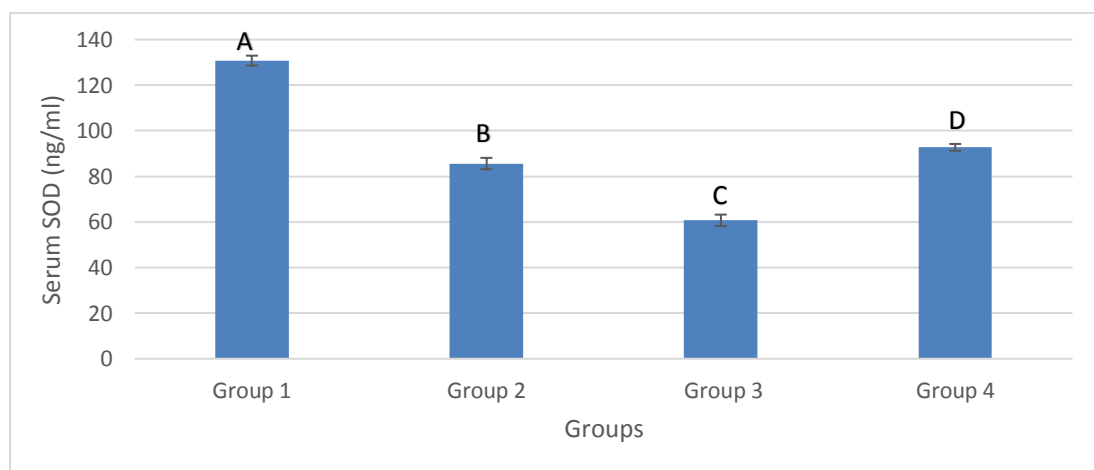


Figure (5): Changes in serum level of SOD.

SOD: Superoxide dismutase. Group 1: control (-ve) group, normal control. Group 2: control positive group. Group 3: Group treated with MTX. Group 4: group treated with combination treatment *C. Chinensis* crude extract and methotrexate. Data expressed as means $\pm$ SEM. Letters with different capital letters is significantly differ. P -value ≤ 0.05 .

Discussion

Chemicals analysis for *C. Chinensis* extract that was performed in this study

demonstrate that the extract was rich with flavonoids, phenolic compounds, steroids,

alkaloids, and other chemicals that have a wide range of pharmacological activities. These findings were agreed with Ye *et al.* and Ruben *et al.* [24].

Flavonoids are a group of naturally occurring substances, found in plant's fruits, roots, stems, and flowers. Flavonoid is well known for its anti-inflammatory property due to its inhibitory effect on COX and lipoxygenase enzymes. Also, flavonoids have anti-oxidant activity by scavenger the free radicals leading to the formation of less reactive compounds [32].

Steroidal compounds that were identified in the extract have an anti-inflammatory effect by inhabiting neutrophil migration, decrease pro-inflammatory cytokines release from macrophage, inhabit NF-Kb activation, and migration and finally promote the release of anti-inflammatory mediators (IL-10) [33].

Alkaloids that also found in *C. Chinensis* have anti-inflammatory effects mediated mainly by inhabiting RANKL pathway [34, 35]. Thiirane is a natural product detected in GC MS of *C. Chinensis* extract. It has an important activity as MMPs inhibitor [36]. Carbamic acid also was identified in GS MS, it has an analgesic effect by its antagonism effect on serotonin receptor (5-HT_{2A}) [37].

According to Vasudevan Aparna *et al.* n-hexadecanoic acid which is a saturated fatty acid has an anti-inflammatory effect by inhabiting PLA2 enzyme and thus suppresses Arachidonic acid synthesis [38].

From the other side, butylated hydroxytoluene has a beneficial effect in decrease fibrosis and slow down the inflammation by its inhibitory effect on TNF- α , TGF- β 1, IL-11 β , IL-8 [39]. Finally, Oleic acid has an important role in immune response. It suppresses the activity of natural killer cells and inhabit secretion and activation of PLA2 enzyme leading to an anti-inflammatory effect [40].

Total body weight was affected in all induced animals due to the effect of inflammatory mediators which increase leptin hormone, elevated level of leptin

lead to decrease body weight as a result of decrease feeding [41].

Animals that received combination treatment in group 4 (*C. Chinensis* and MTX) reflect the preferable weight gain and the mean of animals' weight were not significantly differ (P value > 0.05) from the normal group.

This result may be correlated to a dual inhibitory effect on pro-inflammatory cytokines by MTX and *C. Chinensis* crude extract that leading to decrease inflammatory consequences and improve animal state.

Angiogenesis is an important process in providing new blood vessels to inflamed joints in RA patients. Animals that received CFA in group 2 showed the greatest elevation in serum level of VEGF due to increase its expression locally which related to overproduction of cytokines (TGF- α , TNF- α , IL-1 β , IL-6) by the adjuvant. These cytokines drive the signaling pathways toward the release of pro-angiogenic factors and inhibit anti-angiogenic agents [42].

Methotrexate has an anti-angiogenic effect, when it was used in this experiment animals that had been administered with MTX showed obvious finding in the suppression of serum level of VEGF. The exact mechanism by which MTX inhibit angiogenesis may due to modulation of adhesion cells, or due to inhibition of the conformational fitting of α β γ subunits of VEGF receptors [43].

When combination treatment in group 4 (*C. Chinensis* extract with MTX) were used, serum level of VEGF showed more decrement than MTX treatment, this can be explained by the potent anti-angiogenic effect mediated by MTX and *C. Chinensis* crude extract.

The serum level of MMP₃ was elevated in all induced animals, because of increase its expression in the inflamed joint [44]. When animals were sacrificed at day 35 of the experiment, group 2 showed an increase in serum level of MMP3 to (10.73 $\pm$ 0.28 ng/ml). The *C. Chinensis* extract

when combined with MTX in group 5 show greater inhibition in serum MMP3 due to dual effect, this result was similar to Nagaraja Haleagrahara *et al.* researches in which they found that combination treatment of flavonoid (one of *C. Chinensis* component) with MTX show greater suppression than methotrexate alone ^[45].

C. Chinensis is well-known for its anti-oxidant effect so it may have a beneficial role in RA. Animals treated with MTX show a dramatic decrease in serum SOD and even less than group 2. This can be explained by the effect of oxidative stress that was induced by MTX which led to decrease serum level of anti-oxidant enzymes including (SOD). In human, this inhibition may lead to liver or renal damage especially for patient with aggressive RA, who need high doses from MTX for long periods. This suggestion was agreed with Agman M. Maumout *et al.* ^[47], and agree with M Savvan *et al.* ⁽⁴⁸⁾.

After finalized this experiment, the recommendations were: Repeat this study with a large scale of animals, Fractionate and isolate each chemical entity found in the extract and identify its signaling pathway in RA, Compare the anti-oxidant and anti-angiogenic effect of *C. Chinensis* with other anti-oxidant supplements and anti-angiogenic drugs to assess its effectiveness as a future treatment in RA, and Repeating this study with different doses of MTX and *C. Chinensis* extract to assess the effectiveness of the other doses of combination.

Conclusion

This study had reached to a conclusion that *C. Chinensis* improves objective parameters for RA especially body weight. Methotrexate has a significant beneficial effect in RA, but this effect was augmented when combined with *C. Chinensis* especially in the suppression of serum level of VEGF and MMP₃. The oxidative stress (serum SOD) that was occurred during the treatment of RA with MTX, was

decreased significantly in a treatment protocol that involved the addition of *C. Chinensis* to MTX (increase serum level of SOD).

References

- 1- Zamora NV, Tayar JH, Lopez-Olivo MA, et al. Baricitinib for rheumatoid arthritis (Protocol). *Cochranen Database of Systematic Reviews*. 2019;4: 1-11
- 2- Javaid A, Ibrahim J, Syed Nasir A. B. Rheumatoid arthritis: recent advances on its etiology, role of cytokines and pharmacotherapy. *Biomedicine & pharmacotherapy* 2017; 92:615-633.
- 3- Matthew T, Charles C, Hamel P, et al. Genetic and environmental risk factor for rheumatoid arthritis in UK African ancestry population: the genera case-control study. *Rheumatology* (Oxford). 2017; 56(8): 1282–1292.
- 4- Sarah T A, Sami S, Faiq I G. Efficacy, Safety and Predictors of Response to Rituximab in Treatment of Iraqi Patients with Active Rheumatoid Arthritis. *ANB. MED. J.* 2019; 15(1):16-21.
- 5- Ali M H. Incidence of Rheumatoid Arthritis [2001 to 2011]. *The Iraqi postgraduate medical journal* 2013; 12(4): 568-572.
- 6- Hemalatha A, Nandhini G, Sahanaz P A. Rheumatoid arthritis – a concised review. *Int. J. Sci. Res.* 2019; 8(6): 46-48.
- 7- Yukinori O, Di W, Gosia T, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature*. 2014 Feb 20; 506(7488):376-381.
- 8- Anca I, Vijay J, Lars K, et al. Mechanisms involved in triggering rheumatoid arthritis. *Immunol Rev* 2016; 269(1): 162-174.
- 9- Douglas J, Carl O, Ursula F. Cellular and molecular perspectives in rheumatoid arthritis. *Semin Immunopathol.* 2017; 39(4):343-354.

- 10- Kathleen C, So Min Y, Seong H, et al. Smoking and Rheumatoid Arthritis. *Int J Mol Sci.* 2014; 15(12): 22279–22295.
- 11- Anna I, Lars A, Pernilla W, et al. Occupational and risk of developing rheumatoid arthritis: results from a population –based case- control study. *Arthritis care & research.* 2018; 70(4):499-509.
- 12- Derksen V, Huizinga T, van der D. The role of autoantibodies in the pathophysiology of rheumatoid arthritis. *Semin Immunopathol.* 2017; 39(4):437-446.
- 13- Jeffrey A, Elizabeth W. The roles of cigarette smoking and the lung in the transitions between phases of preclinical rheumatoid arthritis. *Curr Rheumatol Rep.* 2016;18(3):15
- 14- Sasha B, Audrey S, Lawrence J, et al. Industrial air emissions, and proximity to major industrial emitters, are associated with anti-citrullinated protein antibodies. *Environ Res.* 2017; 157:60-63.
- 15- Armando M, Dale P, Laura E, et al. Pesticide exposure and risk of rheumatoid arthritis among licensed male pesticide applicators in the agricultural health study. *Environ Health Perspect.* 2017 14; 125(7):077010.
- 16- Anna I, Lars K, Saedis S, et al. Occupational exposure to asbestos and silica and risk of developing rheumatoid arthritis: findings from a Swedish population-based case-control study. *RMD Open.* 2019; 5: 1-11.
- 17- Markus K, Wolfgang H. Human B cells on their route to latent infection - Early but transient expression of lytic genes of Epstein-Barr virus. *Eur J Cell Biol.* 2012 Jan; 91(1):65-69.
- 18- Lazaros I, Dimitrios D, Stamatis-Nick L, et al. The infectious basis of ACPA-positive rheumatoid arthritis. *Front Microbiol.* 2017 Sep; 8:1853.
- 19- Bae SC, Lee YH. Association between anti-*Porphyromonas gingivalis* antibody, anti-citrullinated protein antibodies, and rheumatoid arthritis: A meta-analysis. *Z Rheumatol.* 2018 Aug; 77(6):522-532.
- 20- Maximilian F, Loreto A, Jesper R, et al. *Aggregatibacter actinomycetemcomitans*-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. *Sci Transl Med.* 2016 Dec 14;8(369):369ra176.
- 21- Stubelius A, Andersson A, Islander U, et al. Ovarian hormones in innate inflammation. *Immunobiology.* 2017 Aug; 222(8-9):878-883.
- 22- Camilla B, Susan M, Cecilia O, et al. Association between menopausal factors and the risk of seronegative and seropositive rheumatoid arthritis: results from the nurses' health studies. *Arthritis care & research.* 2017 Nov; 69(11):1676-1684.
- 23- Shazia N, Sobia N, Shazia A, et al. The genus *Cuscuta* (Convolvulaceae): An updated review on indigenous uses, phytochemistry, and pharmacology. *Iran J Basic Med Sci.* 2019 Nov; 22(11):1225-1252.
- 24- Sineeporn D, Jin L, Yang X, et al. *Cuscuta Chinensis* Lam.: A systematic review on ethnopharmacology, phytochemistry and pharmacology of an important traditional herbal medicine. *J Ethnopharmacol.* 2014 Nov 18; 157:292-308.
- 25- Ateeque A, Sudeep T, Tran D, et al. A review on phytoconstituents and biological activities of *Cuscuta* species. *Biomed Pharmacother.* 2017 Aug; 92:772-795.
- 26- Fadia H, Ibtihal M, Ali H. Studying hypoglycemic activity of *Cuscuta chinensis* Lam. on type 1 Diabetes Mellitus in white male rats. *Journal of Physics: Conference Series.* 2019 Sep; 1294(6):062020.
- 27- Sivapriya T, Sheila J. Identification, isolation and elucidation of

- compounds from fraction of methyl hydroxyl chalcone polymer from aqueous extract of *Cinnamomum Zeylanicum*. *Asian Journal of Pharmacy and Pharmacology*. 2019; 5: 582-588.
- 28- Abeer F, Imad H, Mohanad J. A Review: uses of Gas Chromatography-Mass Spectrometry (GC-MS) technique for analysis of bioactive natural compounds of some plants. *International Journal of Toxicological and Pharmacological Research*. 2017; 9(1); 81-85.
 - 29- Ahmed YM, Messiha BA, Abo-Saif A. Protective effects of Simvastatin and Hesperidin against Complete Freund's Adjuvant-Induced rheumatoid arthritis in rats. *Pharmacology*. 2015; 96(5-6):217-225.
 - 30- Wahba MG, Messiha BA, Abo-Saif AA. Protective effects of fenofibrate and resveratrol in an aggressive model of rheumatoid arthritis in rats. *Pharm Biol*. 2016 Sep; 54(9):1705-1715.
 - 31- Wang X, He X, Zhang CF, et al. Anti-arthritis effect of berberine on adjuvant-induced rheumatoid arthritis in rats. *Biomed Pharmacother*. 2017 May; 89:887-893.
 - 32- Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. *J Nutr Sci*. 2016 Dec 29; 5: e47.
 - 33- Shirishkumar D, Ashwini V, Prashant D. Pharmacological, nutritional, and analytical aspects of β -sitosterol: a review. *Orient Pharm Exp Med*. 2014; 14:193–211.
 - 34- Su-Jin K, Jae-Young U, Ju-Young L. Anti-inflammatory activity of Hyperoside through the suppression of Nuclear factor- κ B activation in mouse peritoneal Macrophages. *Am J Chin Med*. 2011; 39(1):171-181.
 - 35- Jing W, Yu-Tao L, Lu X, et al. Anti-inflammatory effects of Apigenin in lipopolysaccharide-induced inflammatory in acute lung injury by suppressing COX-2 and NF- κ B pathway. *Inflammation*. 2014 Dec; 37(6):2085-2090.
 - 36- Tatyana A, Valery M. The impact factor of the thiirane group in organic compounds on their predicted pharmacological activities: A brief review. *International Journal of Chemical Studies*. 2018; 6(1): 832-839.
 - 37- Chae E1, Yi H, Choi Y, et al. Synthesis and pharmacological evaluation of carbamic acid 1-phenyl-3-(4-phenyl-piperazine-1-yl)-propyl ester derivatives as new analgesic agents. *Bioorg Med Chem Lett*. 2012 Apr 1; 22(7):2434-2439.
 - 38- Vasudevan Aparna¹, Kalarickal V, Pradeep K, et al. Anti-Inflammatory property of n-Hexadecanoic Acid: structural evidence and kinetic assessment. *Chem Biol Drug Des*. 2012; 80: 434–439.
 - 39- Yua L, Yua H, Lianga X, et al. Dietary butylated hydroxytoluene improves lipid metabolism, antioxidant and anti-apoptotic response of largemouth bass (*Micropterus salmoides*). *Fish Shellfish Immunol*. 2018 Jan; 72:220-229.
 - 40- Carrillo C, Cavia M, Alonso-Torre S. Role of oleic acid in immune system; mechanism of action; a review. *Nutr Hosp*. 2012; 27(4):978-990.
 - 41- Aloke C, Ibiam U, Orji O, et al. Anti-arthritis potentials of ethanol and aqueous extracts of stem bark of *Cleistanthus patens* on complete freund's adjuvant-induced rheumatoid arthritis in rats. *J Ayurveda Integr Med*. 2019 Oct 9; S0975-9476(18)30605-3.
 - 42- Hatem A, Zhenlong C, Michael V, et al. The pathogenic role of angiogenesis in rheumatoid arthritis. *Angiogenesis*. 2015 Oct; 18(4): 433–448.
 - 43- Shaker OG, Khairallah M, Rasheed HM, et al. Antiangiogenic effect of methotrexate and PUVA on psoriasis.

- Cell Biochem Biophys. 2013 Nov; 67(2):735-742.
- 44- Yosuke H, Daihei K, Atsushi K. Normal serum matrix metalloproteinase-3 levels can be used to predict clinical remission and normal physical function in patients with rheumatoid arthritis. Clin Rheumatol. 2017 Jan; 38(1):181-187.
 - 45- Nagaraja H, Kelly H, Socorro MH, et al. K. Flavonoid quercetin–methotrexate combination inhibits inflammatory mediators and matrix metalloproteinase expression, providing protection to joints in collagen-induced arthritis. Inflammopharmacology. 2018; 26: 1219-1232.
 - 46- Yan L, Yang X, Weidan J, et al. Anti-tumor activities of Matrine and Oxymatrine. Tumour Biol. 2014 Jun; 35(6):5111-5119.
 - 47- Mahmoud AM, Hussein OE, Hozayen WG, et al. Methotrexate hepatotoxicity is associated with oxidative stress, and down-regulation of PPAR γ and Nrf2: Protective effect of 18 β -Glycyrrhetic acid. Chem Biol Interact. 2017 May 25; 270:59-72.
 - 48- M Savran, E Cicek, DK Doguc, et al. Vitamin C attenuates methotrexate-induced oxidative stress in kidney and liver of rats. Physiology International. 2017; 104 (2): 139–149.